ERRATUM.

Isolation and Properties of the Factor Responsible for Increased Capillary Permeability in Inflammation, by Valy Menkin, 1937, **36**, the average figures on p. 166, refer to total protein in grams per 100 cc. of serum and of exudate respectively; and not to "total protein nitrogen" as stated in the article.

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9186 P

Immunological Relationships of Encapsulated Gram-Negative Rods.*

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The difficulties encountered in the past, in the differentiation of the bacteria exemplified by the organisms of Friedländer, rhinoscleroma, ozaena, granuloma inguinale and *Bact. aerogenes,* have more recently been increased, rather than diminished, by the discov-

* This work was conducted under a grant from the Commonwealth Fund of New York.

TABLE I.

Summary of Agglutination of "R" Cells from Encapsulated Gram-Negative Rods.

"R" Antigen derived from	Antisera (1:10 dilution)										
	Friedländer			Rhino-scleroma	Ozaena			Aerogenes			Granu-loma
	A	C	X		I	II	III	No. 1	No. 11	No. F8	
Friedländer											
Type A	++++	++++	++++	—	—	—	—	—	—	—	—
Type C	++++	++++	++++	—	—	—	—	—	—	—	—
Group X	++++	++++	++++	—	—	—	—	—	—	—	—
Rhinoscleroma	—	—	—	++++	++++	++++	++++	++++	++++	++++	++++
Ozaena											
Type I	—	—	—	++++	++++	++++	++++	++++	++++	++++	++++
Type II	—	—	—	++++	++++	++++	++++	++++	++++	++++	++++
Group III	—	—	—	++++	++++	++++	++++	++++	++++	++++	++++
Aerogenes											
No. 1	—	—	—	++++	++++	++++	++++	++++	++++	++++	++++
No. 11	—	—	—	++++	++++	++++	++++	++++	++++	++++	++++
No. F8	—	—	—	++++	++++	++++	++++	++++	++++	++++	++++
Granuloma											
No. 8	—	—	—	++++	++++	++++	++++	++++	++++	++++	++++

ery that members of the different species possess common immunological characteristics by virtue of similarities in the capsular carbohydrate.[1] Studies on the immunological reactions of the unencapsulated cell[2] supplied the hypothesis that the different organisms, once deprived of the ability to elaborate capsular polysaccharide, might be more readily amenable to systematization. Accordingly, unencapsulated, or "R", strains have been derived from the encapsulated "S" strains by continued cultivation of the "S" form in homologous anti-S serum. Usually, 6 to 12 transplants in broth containing 10% immune serum sufficed for the conversion. Rabbits were then injected intravenously with suspensions of heat-killed (30 min. at 56°C.) R forms until the sera contained sufficient antibody for serological study.

For this purpose, anti-sera were obtained with R cells derived from Friedländer's bacillus, Types A and B and Group X; ozaena bacillus, Types I and II and Group III; three immunologically different strains of *Bact. aerogenes;* and a single strain of rhinoscleroma and granuloma bacillus. Agglutinations were then conducted employing each R strain and each anti-serum. In order to illustrate the reactions observed, a summary protocol has been arranged of all the reactions. The titres of the different anti-sera were close to 1:5000, and the agglutination was composed of large, coarse granules readily disrupted on agitation. This is, of course, in contradistinction to the agglutination of S cells, which is characterized by a compact, tenacious disc. Examination of the data reveals 2 large groups disclosed by the agglutination reaction. The Friedländer bacilli comprise one group, while the rhinoscleroma, ozaena, aerogenes and granuloma organisms constitute the second group.

It will be observed that no data are presented for the reactions of Type B Friedländer's bacillus. These have been omitted intentionally, since experiments with this organism and its relation to *Bact. aerogenes* are still in progress. The only statement to be made at the present time is that several strains, originally identified in other laboratories, as well as our own, as Type B Friedländer's bacillus, are now recognizable as strains of *Bact. aerogenes.*

In any case, the conclusion seems obvious that by agglutination of R variants, the encapsulated, gram-negative rods may be

[1] Small, J. C., and Julianelle, L. A., *J. Inf. Dis.*, 1923, **32**, 456; Avery, O. T., Heidelberger, M., and Goebel, W. F., *J. Exp. Med.*, 1925, **42**, 709; Edwards, P. R., *J. Bact.*, 1929, **17**, 339; Goslings, W. R. O., Thesis, Univ. Amsterdam, 1933; also *Cent. Bakt.*, 1936, **136**, 1; Morris, M. C., and Julianelle, L. A., *J. Inf. Dis.*, 1934, **55**, 150.

[2] Julianelle, L. A., *J. Exp. Med.*, 1926, **44**, 683; Goslings, W. R. O., *vid.* Ref. 1.

classified into 2 large groups—composed, in one instance, of all the Friedländer bacilli, and in the other, of the organisms of rhinoscleroma, ozaena, granuloma inguinale and *Bact. aerogenes.* Whether the serological distinctions indicate that Friedländer bacilli arise genetically from one source, and the remaining organisms from a second and different source, remains for future investigation to solve.

9187 P

Modification by Strychnine of Response of the Optic Cortex.*

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Stimulation of the optic nerve of the rabbit by a single shock results in a sequence of potential changes recorded from the optic cortex which occupy 1/5 second. At threshold for the response, the first potential is a monophasic surface-positive wave, followed by a surface-negative, and this in turn by a surface-positive deflection. Above threshold, the initial positive wave is covered up by a larger diphasic response (Bartley and Bishop[2]). Ether depresses this diphasic response, and strychnine increases it differentially (Bartley[1]), as compared to the sequence initiated by the monophasic wave. Dilute strychnine applied locally to the cortical surface may increase the diphasic component by 500% without any change in the amplitude of later parts of the record. Higher concentrations depress the late components to extinction, and increase the diphasic wave still further. Finally, spontaneous responses occur, first singly, then in trains. Whether recorded from across the whole cortex, or from needles subtending only certain strata, this spontaneous wave duplicates the diphasic response to stimulation both in relative amplitude of the 2 phases and in duration. The spontaneous rhythmic activity which is present before the application of strychnine, decreases and disappears parallel with the disappearance of the later components of the stimulated response. Facilitation to a second response, which accompanies the late surface-positive wave of

* Aided by a grant from the Rockefeller Foundation for Research in neurophysiology.

1 Bartley, S. H., *Am. J. Physiol.*, 1933, **103**, 203.

2 Bartley, S. H., and Bishop, G. H., *Am. J. Physiol.*, 1933, **103**, 159.

the normal record, disappears as this component is suppressed by strychnine.

Strychnine thus serves to differentiate 2 sequences of potential, one consisting of the 2 phases of the early diphasic component, lasting about 20 ms., the other consisting of the triphasic sequence of 3 slow waves lasting 200 ms. The latter can be correlated with the normal spontaneous 5 per second rhythm in a number of respects (Bishop[3]), to the extent that it has been inferred to occupy the same cortical elements. It is presumably not the immediate correlate of vision, both because it it not suppressed by a degree of narcosis that would abolish visual function, and because the spontaneous activity goes on even after cutting, and in fact after degeneration of the optic nerve. The former diphasic process is inferred to be the immediate visual one. Its marked increase by strychnine corresponds to the increased functional excitability of spinal cord and motor cortex under this drug, as indicated by peripheral reflex and motor effects.

Recorded from pairs of needle electrodes subtending different strata of the cortex, the potentials observed indicate that these two processes take different routes. The first potential of the diphasic process indicates that the middle strata are negative to those above, while the second potential indicates that the upper strata are negative to the lower, or in some cases both upper and lower negative to the middle. The progress of the activity represented by this would therefore seem to be mainly from the middle layers of the cortex upward, to discharge via the plexiform white matter, and perhaps also via the subcortical white matter. The triphasic process similarly originates in the middle or lower cortical layers, and progresses upward, but during the third potential of this series occupies the whole cortex, the greatest negativity being toward the deepest strata. It is inferred to involve the discharge of pyramid cells whose axons leave the cortex via the white matter below the 6th layer.

This analysis applies only to what may be termed the mass-impulse, and not to the discharges of single elements. That is, while the mass impulse represented by the diphasic process appears to discharge in general upward toward the surface white matter, it cannot be said that no discharge takes place during this time in the reverse direction, or laterally, but only that the center of greatest activity as indicated by vertically oriented electrodes progresses upward. Similarly, during the third potential of the triphasic series,

[3] Bishop, G. H., *Cold Spring Harbor Symposium*, 1936, **4**, 305.

an increasing negativity from the surface to the subcortical white matter suggests a predominance of downwardly directed impulses. The facilitation to a second stimulus accompanying this potential presumably involves a cortico-thalamic discharge.

9188

Effects of Sugar, Glycerin and Urea on Hormones of Cattle Anterior Pituitary Glands.*

S. J. HAYWARD AND LEO LOEB.

From the Department of Pathology, Washington University School of Medicine, St. Louis, Mo.

In a series of former investigations we have distinguished essentially 3 types of effects of anterior pituitary glands on the ovary: (1) The destruction of follicles; (2) various luteinization processes, and (3) the full growth and maturation of follicles, followed in some cases by ovulation and formation of true corpora lutea. We also studied the relation of the thyroid-stimulating hormone to the substances affecting the ovaries. At first we noticed a close association between the thyroid-stimulating hormone and the substances inducing atresia and luteinization processes in the ovary. We considered it, therefore, probable that the thyroid-stimulating hormone and atresin, which is responsible for the destruction of ovarian follicles, were one and the same substance.[1] However, subsequently we developed a method which made possible the analysis of the effects produced by the anterior pituitary glands of various species by subjecting these organs *in vitro* to the action of various solutions.[2] The results of these experiments indicated that the effects of hormones on the thyroid gland were distinct from those acting on the ovary. In the present investigation we extended the application of this method by immersing the anterior pituitary glands of cattle in glycerin and in solutions of cane sugar and urea before transplanting them subcutaneously into guinea pigs. One cattle gland, or parts of a cattle gland were immersed in 80 cc. of the fluid for various periods of

* These experiments were carried out with the aid of a grant from the International Cancer Research Foundation and of a grant for research in science made to Washington University by the Rockefeller Foundation.

[1] Loeb, Leo, Volume jubilaire dédié an Prof. Lina Stern, Moscow, 1935, p. 405.

[2] Loeb, Leo, Anderson, H. C., Saxton, John, Hayward, S. J., and Kippen, A. A., *Science*, 1935, **82**, 331.

time. One-fourth of a gland was implanted daily, on 4 consecutive days, into immature female guinea pigs weighing approximately 175-180 gm. Previous to transplantation the gland was freed from adhering solution by rinsing it in sterile 0.9% NaCl solution. If fresh, non-treated cattle anterior pituitary is thus implanted and examination of the guinea pig is made on the day following the fourth implantation, it is found that atresin effects, in some cases accompanied by slight luteinization processes, predominate.

I. *Experiments with cane sugar.* (12 experiments). In most experiments saturated cane sugar solutions, and in a few cases 20% solutions were used. The glands were left in the different solutions for one hour, 4 hours, one, or 2 days. Some of the glands which were left in for one hour, and a few of the others, produced mainly atresin effects together with a slight theca luteinization, such as formation of interstitial gland in the medulla of the ovary and a mild luteinization of theca in quite atretic follicles; in one case also pseudolutein bodies developed. In the other cases various combinations of theca and granulosa luteinization predominated. In addition to the above named luteinization changes, luteinization in the theca of smaller preserved follicles, formation of lutein rings during the stage of early connective tissue atresia and of luteinizing connective tissue atresia occurred. Also interstitial gland bodies and transitions between some of these formations were occasionally noted. There was, as a rule, a definite hypertrophy of the thyroid gland.

The characteristic effect of these solutions consists in the preservation of those substances in the treated gland which produce the various luteinizations.

II. *Experiments with glycerin.* (11 experiments.) In the majority of cases pure glycerin, but in a few instances 50% glycerin, was used. The anterior pituitary glands were left in the solutions 4 hours, one, 2, or 3 days. The results were essentially the same as with cane sugar. In a few cases, especially in the experiments with shorter immersion of the glands in the solution, atresin effects together with slight luteinization were observed, but as a rule, the various combinations of theca and granulosa luteinization predominated. In addition to the above named changes in a few cases a premature maturation or luteinization of the granulosa of smaller, preserved follicles was seen, and in one case we observed ingrowth of connective tissue into the not enlarged granulosa, which was thus divided into parcels. In the majority of cases the thyroid gland showed much hypertrophy, but in some experiments this was only moderate.

III. *Experiments with urea solutions.* (66 experiments.) Saturated, as well as weaker solutions of urea (up to 10%) were used; in the majority of the experiments the solution contained 50% urea; the solutions with the immersed glands were kept mostly at room temperature, but some at 37°C., and others at 40°C., for one, 4, 10, 15 hours, one, 2, 3, or 4 days. The results differed in this series quite markedly from those obtained in Series I and II. The luteinization changes which were characteristic of sugar and glycerin action, were rather inconspicuous in the case of urea solutions; the latter produced principally maturation of follicles without any other changes in the ovaries, but sometimes also pseudolutein bodies, due perhaps in certain instances to an ingrowth of connective tissue and vessels into the mature granulosa; other luteinization processes were rare and slight. Maturation processes in full-sized follicles were found in 26 animals, that is, in more than one-third of all the guinea pigs. In these experiments the urea had caused to a large extent the elimination of the substances responsible for atresin action and luteinization, especially theca luteinization. In 22 cases the action of the urea solution went still further, leading to the entire elimination of all the hormones acting on the ovaries. In the remaining experiments, mainly pseudolutein bodies, either with or without other luteinization products, developed; in some cases the animals were sick and thus hypotypical ovaries were produced. In a few instances atresin action was noted, especially in some experiments in which the glands had been left only for one hour in the urea solutions. The greatest number of mature follicles was observed after an immersion for from 10 hours to 2 days in the solution. After 3 days, the ovaries in which mature follicles were observed were fewer, while those in which no hormone effects were seen, had increased in number. After 4 days, large mature follicles, without any other changes, were found. Of special interest is one case in which mature follicles had just ruptured and in which, in addition, a pseudolutein body was present. However, usually pseudolutein bodies developed as the result of ingrowth of connective tissue and blood vessels into the mature or maturing granulosa of large follicles.

As to the thyroid gland, in the large majority of cases in which fully mature follicles were found in the ovaries, the thyroid-stimulating hormone had been eliminated; but in some of these animals the thyroid showed moderate or even more marked hypertrophy. Also in those guinea pigs, in which the treated anterior pituitary glands no longer exerted any hormone effects on the ovary, thyroid stimulation was lacking in the large majority of cases; but moderate hypertrophy was found in some of the experiments. Thyroid stim-

ulation was more frequently associated with the third group of experiments, in which granulosa luteinization (pseudolutein bodies) or atresin effects predominated. We may then conclude that the thyroid-stimulating hormone, while in many cases associated with theca luteinization or atresin effects, is distinct from the hormone producing these ovarian effects. This conclusion is in agreement with views previously expressed by us.[3]

Summary. 1. When the anterior pituitary glands of cattle are kept *in vitro* in cane sugar solutions or in glycerin, luteinization processes in theca and granulosa predominate; but if the glands are left in the solution for only one hour atresin effects may still be noticeable. Thyroid hypertrophy is found in the majority of cases. 2. When, instead of cane sugar or glycerin, solutions of urea are used, formation of mature follicles is induced by the glands thus treated in more than one-third of the cases. In another third the action of the urea was so strong that all the ovarian hormones were eliminated. In the majority of these animals also the thyroid hormone had been lost, but in some cases the formation of mature follicles was associated with thyroid hypertrophy. In somewhat less than one-third of the cases formation of pseudolutein bodies, with or without other luteinization processes, and in a few cases atresin effects were observable. 3. While thus glycerin and cane sugar solutions make possible the production of luteinizing effects by the implanted glands, after application of urea solutions only maturation processes are produced, or luteinization effects mainly of the granulosa, or in still other cases all the hormones are eliminated.

9189 P

Relationship Between Blood Amylase and Urinary Amylase in Man.

S. H. GRAY AND MICHAEL SOMOGYI.

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Somogyi[1] has shown that the amylase content in the blood is rather constant for the individual, while the variations from individual to individual are considerable. The study of the amylase content of the urine, however, reveals great irregularity in the same individual at various periods of the day, without any apparent regu-

[3] Loeb, Leo, Saxton, John, and Hayward, S. J., *Endocrin.*, 1936, **20**, 511.

[1] Somogyi, Michael, PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 538.

larity in the variations. The analytical methods used were those described by Somogyi.[1, 2, 3] We found it very important to take into consideration the optimum pH (6.8-7.4) and the optimum salt concentration (0.25-0.4% NaCl).

In healthy human beings the concentration of the enzyme in the urine is greater than in the blood, the ratio of urine amylase to blood amylase being usually between 2:1 and 6:1. These fluctuations in ratio occur in the same person, often in the course of a single day. Great as these variations are, we find that on the whole there is a parallelism between the blood and urinary amylase concentrations; *i. e.*, high blood amylase usually goes with a high urinary amylase, and low blood amylase with a low urinary amylase.

In pancreatic injury, as acute pancreatitis, obstruction of pancreatic ducts or trauma of the gland, there is a tremendous increase in both amylases, but without a shift in the relative concentrations. Since the urinary amylase remains high for a period of about 24 hours longer than the blood amylase, it may be preferable to determine the urinary amylase for the diagnosis of acute pancreatitis.

In conditions where the blood amylase is low, as in severe toxemias of pregnancy, pneumonia, liver abscess, many cases of cholecystitis, obstructive jaundice and diabetes,[1] the urinary amylase is proportionately low. Both occasionally may be zero. Thus, although in these conditions there are extremes of amylase concentration, the ratios between the urine and blood remain the same as in healthy human beings.

On the other hand, there are conditions in which the ratio is markedly reduced to such a degree that the blood amylase concentration exceeds the urinary amylase concentration, giving a ratio of less than one. The blood amylase in such conditions may be subnormal, normal, or definitely above normal. If moderate elevations in blood amylase occur, it is necessary to determine urinary amylase in order to differentiate between kidney retention and pancreatic disturbance as the causative factor.

Reversal of the urinary amylase/blood amylase ratio was observed mainly in kidney disturbances. Our series, as yet small, includes cases of acute and chronic glomerulonephritis, amyloid nephrosis and lipoid nephrosis.

We have attempted to study the amylase excretion in cases of scarlet fever, a disease where acute nephritis is most frequently

2 Somogyi, Michael, PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1126.

3 Somogyi, Michael. Both methods in detail will be published in the near future.

found as a complication. We wished to obtain the blood-urinary amylase ratios before nephritis set in, so that we could study the progressive change in the ratios from the incipiency of the kidney lesion. During the period of this study none of these cases developed acute nephritis. Yet, in 26 of the 35 scarlet fever patients there was a reversal of the ratio, suggesting that scarlet fever is usually accompanied by kidney damage of a degree insufficient to be observed clinically. At the same time, 18 cases of diphtheria and measles were studied; of these only 4 showed a ratio slightly below one.

Work is in progress to evaluate the amylase clearance as a possible kidney function test.

9190 P

Effect of Liver Extract on Thyroid Glands of Mice and Guinea Pigs.

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During experiments to test the effect of prolonged administration of liver extract on the blood-forming organs, attention was drawn to the thyroid gland by the hyperactivity and excitability of the animals so treated.

The thyroid glands of 20 mice, injected daily with from 0.1 to 0.5 cc. of concentrated liver extract* for from 14 to 480 days, showed changes after 6 or 7 weeks of injection which progressed slowly but steadily as long as the injections were continued. These alterations consisted of: (1) Localized degeneration of several epithelial cells in some of the acini. (2) A general lowering of the height of the alveolar epithelium, associated with an increase in the amount of colloid contained in the acini. (3) Rupture of acini due either to pressure of an increased content of colloid inside the acinus or to isolated degeneration of the alveolar cells. (4) Accumulation of colloid material in the inter-acinar spaces, causing additional injury as well as compression, distortion, and separation of the acini. (5) A gradual increase in the amount of fibrous tissue in the stroma of the thyroid gland.

* Solution Liver Extract, Concentrated, very generously supplied by Eli Lilly and Co.

No very definite changes have been noted in the thyroid glands of 6 guinea pigs injected daily for from 9 to 60 days, with from 0.5 to 1.0 cc. of concentrated liver extract. Whether injections for longer periods, or of larger doses, will produce alterations similar to those noted in mice is under investigation at present.

While uninjected control mice showed none of the abnormalities noted in the experimental animals, it seemed possible that the thyroid changes produced by liver extract might be due to non-specific reactions which could also be elicited by extracts of other organs. Accordingly, a control extract was prepared from beef muscle, using the same method as that described for the preparation of liver extract.[1] To date, mice injected up to 90 days with this control extract have been studied. The changes produced in the thyroid gland are similar in character to those produced by liver extract, but they are considerably less in degree. It must be pointed out, therefore, that the thyroid changes described in these experiments cannot as yet be attributed to any specific substance in liver extract. It is quite possible that the difference in reaction between animals injected with control solution and those injected with liver extract are due to differences in the concentration of non-specific protein materials in the 2 solutions, or to some other non-specific factor. We hope to undertake experiments soon which will decide this point.

9191 P

Studies in Tissue Pressure.

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Although the part played by tissue pressure in the control of the movement of fluid between blood vessels and tissue spaces is but vaguely understood, recent studies appear to demonstrate that it is a significant factor.[1, 2, 3] The wide variations in subcutaneous tissue

[1] Liver Extract, Lilly, Concentrated solution, *N. N. R.*, 1936, p. 282.

[1] Landis, E. M., and Gibbon, J. H., *J. Clin. Invest.*, 1933, **12**, 105.

[2] Youmans, J. B., Wells, H. S., Donley, D., and Miller, D. G., *J. Clin. Invest.*, 1934, **13**, 447.

[3] Weech, A. A., Goettsch, E., and Reeves, E. B., *J. Soc. Exp. Biol. and Med.*, 1934, **60**, 63.

pressure found in the literature[4, 5, 6] have prompted us to determine directly the tissue pressure by the simple manometric method employed by Henderson[7] in the determination of muscle tone. A 26 gauge needle was used in place of a 20. In each determination 3 readings were taken which agreed within 2 mm. of water. In this report we are presenting the results in normal subjects. The results in patients with edema will be presented later.

TABLE I.
Subcutaneous Tissue Pressure at Heart Level in Millimeters of Water.

Subject No.	Age	Sex	Dorsum of Hand	Volar Surface Forearm	Pretibial Area	Dorsum of Foot
1	23	M	28	11	22	35
2	49	M	12	29	22	36
3	24	M	19	26	23	24
4	25	F	17	23	54	15
5	25	M	8	40	54	43
6	29	M	15	19	45	36
7	27	F	22	18	40	38
8	23	M	14	17	18	18
9	26	M	14	14	43	25
10	48	M	30	39	50	38
Mean			17.9	23.8	37.1	30.8

TABLE II.
Subcutaneous Tissue Pressure of the Dorsum of the Foot in Millimeters of Water.

Subject No.	Age	Standing without wt. bearing	Standing with wt. bearing (Needle removed and reinserted)
11	33	76	100
12	23	31	38
13	26	66	82
14	26	42	102
Mean		53.9	80.5

The results are summarized in Tables I, II and III. The mean values at heart level varied from 17.9 to 37.1 mm. of water. The tissue pressure in the lower extremity was increased in the erect position, and further increased by weight bearing. Repeated determinations were made in one subject in the dorsum of the foot at heart level with withdrawal of the needle for each determination. The values obtained were 43, 30 and 38 mm. of water. Six subjects stood for one hour against a table inclined at 75°. The tissue pres-

4 Landerer, A., *Die Gewebesspannung*, Leipzig, 1884.

5 Gildemeister, M., and Hoffmann, L., *Pfluger's Arch.*, 1922, **195**, 153.

6 Meyer, F., and Holland, G., *Arch. f. Exp. Path. und Pharmakol.*, 1932, **168**, 581.

7 Henderson, Y., Ongleterson, A. W., Greenberg, L. A., and Searle, C. P., *Am. J. Physiol.*, 1935-36, **114**, 261.

sure readings in the dorsum of the foot at the beginning of the hour varied from 22 to 94 mm. of water, at the end of the hour 34 to 104 mm. of water with mean values of 53.7 mm. and 65.2 mm. respectively. The maximum increase in value after the one hour period was 31 mm. of water. In none of our subjects in the standing position even up to an hour did the readings approach the higher values found in the literature.

The effect of venous pressure on tissue pressure readings was determined in the normal subjects. The venous pressure was increased by inflating a blood pressure cuff around the arm in steps of 135 mm. of water (10 mm. of mercury) at 2 minute intervals. In no case was the diastolic blood pressure exceeded. The tissue pressure readings made in the dorsum of the hand are illustrated in Table III. Increase of venous pressure over short intervals of time had relatively slight effect on the tissue pressure readings.

TABLE III.
The Effect of Venous Pressure on Tissue Pressure Determination in 10 Subjects.

Pressure in cuff constricting arm mm. water	Mean estimated tissue pressure mm. water
0	20.3
270	19.0
405	19.4
540	21.3
675	24.6
810	27.1

Summary. With the use of a manometric method for direct determination of tissue pressure it was found that the mean subcutaneous tissue pressure in the extremities at heart level varied between 17.9 and 37.1 mm. of water. The subcutaneous tissue pressure was increased in the foot on assuming the erect position and further on weight bearing. Factors other than venous pressure are important in the regulation of tissue pressure.

9192

Studies in the Pathology of Blood Vessels in Man. IV. Volume Changes in Human Finger-Tip Following Sudden Venous Obstruction.*

William A. Sodeman, George E. Burch and Roy H. Turner

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During observations on the rate of blood flow through the finger tip with the use of a sensitive sphygmoplethysmograph,[1] certain results were noted which led to these studies. A cellulose acetate cup without supporting stays was used in place of the one described.[1] Immediately proximal to the cup an occluding air cuff was loosely wrapped so as not to produce any constriction when deflated. The cuff was connected to a pressure reservoir and manometer so that any desired pressure might be suddenly applied. Thirteen normal subjects, ranging from 22 to 52 years of age, were studied under controlled atmospheric conditions (75 ± 1°F. and 50 ± 5% relative humidity). They were seated comfortably with the arm resting passively upon a support with the finger-tip at heart level, 30 minutes being allowed to reach a steady metabolic state. Reactions were then noted for obstructing pressures varying from 5 mm. Hg. to diastolic pressure and maintained for 15 to 120 seconds, an interval exceeding the time of obstruction being allowed for recovery between each observation.

For cuff pressures sufficient to cause swelling of the finger-tip and up to a certain critical level, varying for different persons from 15 to 40 mm. Hg. (Table I) there occurred not only the primary swelling of the finger-tip but also a spontaneous secondary diminution in volume (Fig. 1-A). But when cuff pressures above this level were applied only swelling occurred (Fig. 1-B).

The gain in volume of the finger-tip immediately following application of pressure in the obstructing cuff is probably due mainly to distension of small veins, venules and capillaries which are the vessels known to distend to the pressures employed, and the loss of volume which followed under proper conditions is probably due to active constriction of these same vessels. Further evidence to show that they were active in this response is the fact that after the volume had again reached its former level without a change of intravenous

* Aided by grants from the David Trautman Schwartz Research Fund, the Committee on Scientific Research of the American Medical Association, and the Josiah Macy, Jr., Foundation.

1 Turner, R. H. To be published.

TABLE I.

Volume changes in the finger-tip following the application of the maximum obstructing pressure following which there was a secondary diminution in volume. Obstructing pressure was usually released as soon as the volume returned to the original value.

Subject No.	Age	Sex	Blood pressure	Pressure in obstructing cuff, mm. Hg.	Immediate increase in vol. following obstruction, cu.mm.	Secondary decrease in vol., cu.mm.	Further decrease in vol. on release of obstruction, cu.mm.
1	27	F	96/68	30	—*	—	—
2	26	M	112/80	20	26.0	33.0	47.2
3	22	M	108/78	35	36.4	46.8	55.6
4	26	M	114/88	30	50.7	50.7	80.2
5	25	M	110/72	20	12.8	17.8	25.9
6	22	M	118/82	15	22.5	31.5	12.5
7	24	M	118/74	15	—*	—	—
8	26	F	114/90	15	13.4	13.4	34.0
9	26	M	110/82	35	26.2	26.2	52.0
10	26	F	108/68	25	16.2	21.6	11.4
11	42	M	116/94	17.5	14.0	14.0	16.7
12	45	M	118/84	40	40.4	40.4	50.0
13	52	M	100/62	15	19.2	19.2	22.3
Mean				24	25.2	28.6	37.1
Max.				40	50.7	50.7	80.2
Min.				15	12.8	13.4	11.4

* Reaction occurred but accurate quantitative data were not available.

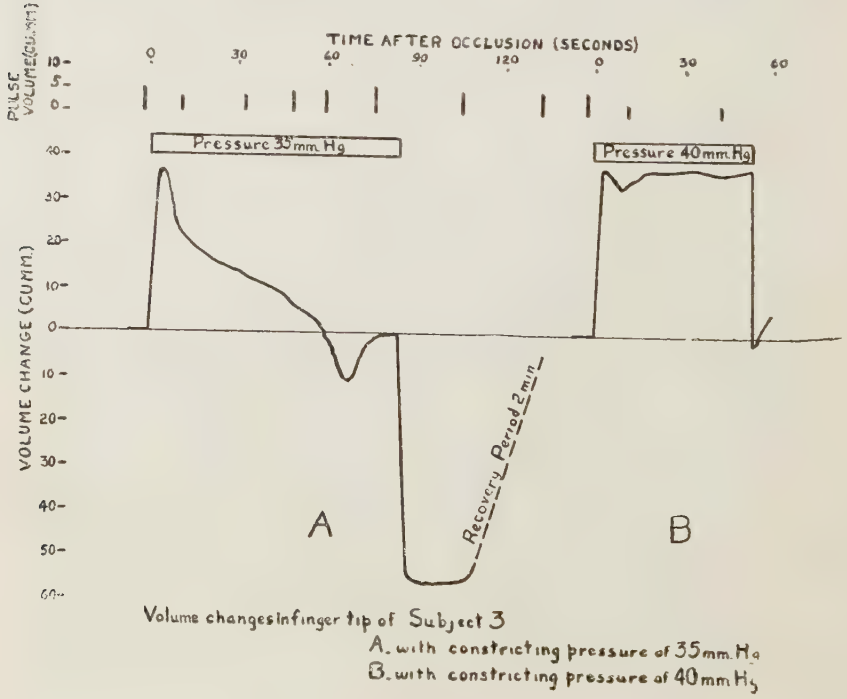

FIG. 1.

pressure sudden release of the constricting pressure in every instance produced an immediate diminution of finger-tip volume below the original level and usually greater than the volume increase resulting from occlusion. The volume loss may be due entirely to primary, active constriction of the non-arterial vessels or in part to constriction of arterial vessels which could through diminution of blood flow within the capillaries and venules allow a passive constriction of these small vessels. We believe that the constriction is predominantly a non-arterial phenomenon.

The critical pressure at which the type of response changes is an index of tone of the vessels responsible for the volume change at that time. The mechanism of the response is not known but it is likely that it is initiated by increased intravascular tension. That local constricting pressure without venous distension is not an adequate stimulus is evidenced by lack of response to sudden momentary pressure in the cuff. Active responses of veins to other stimuli have been reported by others.[2–5]

These phenomena are being studied further in normal and diseased individuals.

Summary. Secondary spontaneous diminution of volume of the finger-tip following sudden partial obstruction of the venous flow from the part is reported. The reaction seems to be due to active constriction of veins, venules and capillaries which may be initiated by increased tension within these vessels.

9193

Sympathetic Ganglion Cell Changes in Adrenalectomized Animals.

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Recent literature is replete with reports of the functional disturbances in animals deprived of their adrenal glands but offers little

2 Hooker, D. R., *Physiol. Rev.*, 1921, **1**, 112.

3 Krogh, A., *The Anatomy and Physiology of Capillaries*, New Haven, 1929, 2nd Ed.

4 Lewis, T., *The Blood Vessels of the Human Skin and Their Responses*, London, 1927.

5 Capps, R. B., *J. Clin. Invest.*, 1936, **15**, 229.

information as to accompanying structural changes. In view of the well-established physiological relationship between this gland and the autonomic nervous system the authors undertook a preliminary study of sympathetic ganglion cells in adrenalectomized cats with a view to determining what structural changes, if any, accompanied the adrenal insufficiency.

The procedure was as follows: The right adrenal gland was removed by a retroperitoneal approach and 7 to 10 days later the left gland was removed in a similar manner. Both operations were done under intraperitoneal nembutal anesthesia. The cats utilized for the study lived from 3 to 12 days following completion of the adrenalectomy. Five of these cats died of adrenal insufficiency and the remainder were killed by opening the thorax and abdomen under ether anesthesia and eviscerating the animal. The superior cervical and stellate ganglia only were removed in the first 6 animals but in the remainder the coeliac and the thoraco-lumbar chain ganglia were also removed. The material was fixed in formalin-acetic acid, sectioned in paraffin, and alternate sections stained with cresyl violet and iron haematoxylin.

Later, a series of experiments was designed to substantiate the preliminary findings as well as to provide for controls. Twelve male cats were kept for a period of 5 weeks, during which time they were given a vermifuge and fed on a well-balanced diet. All were subjected to the same conditions and all were in excellent nutritive state at the end of the 5 weeks. Six of the cats were then adrenalectomized as in the preliminary series. When one of the operated animals was autopsied a normal one was also killed and corresponding ganglia from the 2 cats were carried through the fixative, dehydrative fluids, paraffin, etc., in the same containers. Finally, sections of ganglia from the normal and from the operated animal were mounted on the same slide and thus stained simultaneously. This series disposed of any question of misinterpretation of our findings in the preliminary series due to possible alterations of cell structure by the technical procedure to which the preparations were subjected. It also answers the question of the effect of etherization on the Nissl substance since the normal animal required much more ether at autopsy than did the operated one.

An additional precaution may be mentioned by way of ruling out the possibility of the changes described being attributed to axon injury. Ganglia from 2 cats in which an attempt was made to destroy all nerve fibers in the region of the adrenals were examined as well as ganglia from animals which had been subjected to unilateral adrenalectomy only. In both cases all ganglia appeared normal.

Further controls indicated and carried out were as follows: Ganglia were examined from cats in various stages of inanition and from one cat which had been starved. Material was secured from a number of animals at varying intervals post-mortem. One cat was killed and kept at room temperature for 12 hours, during which time a ganglion was removed at 2-hour intervals. Other animals killed by repeated doses of nembutal and by unintentional overdose of the same drug were examined. In none of these cases were changes present of the nature of those found in the ganglia of adrenalectomized animals.

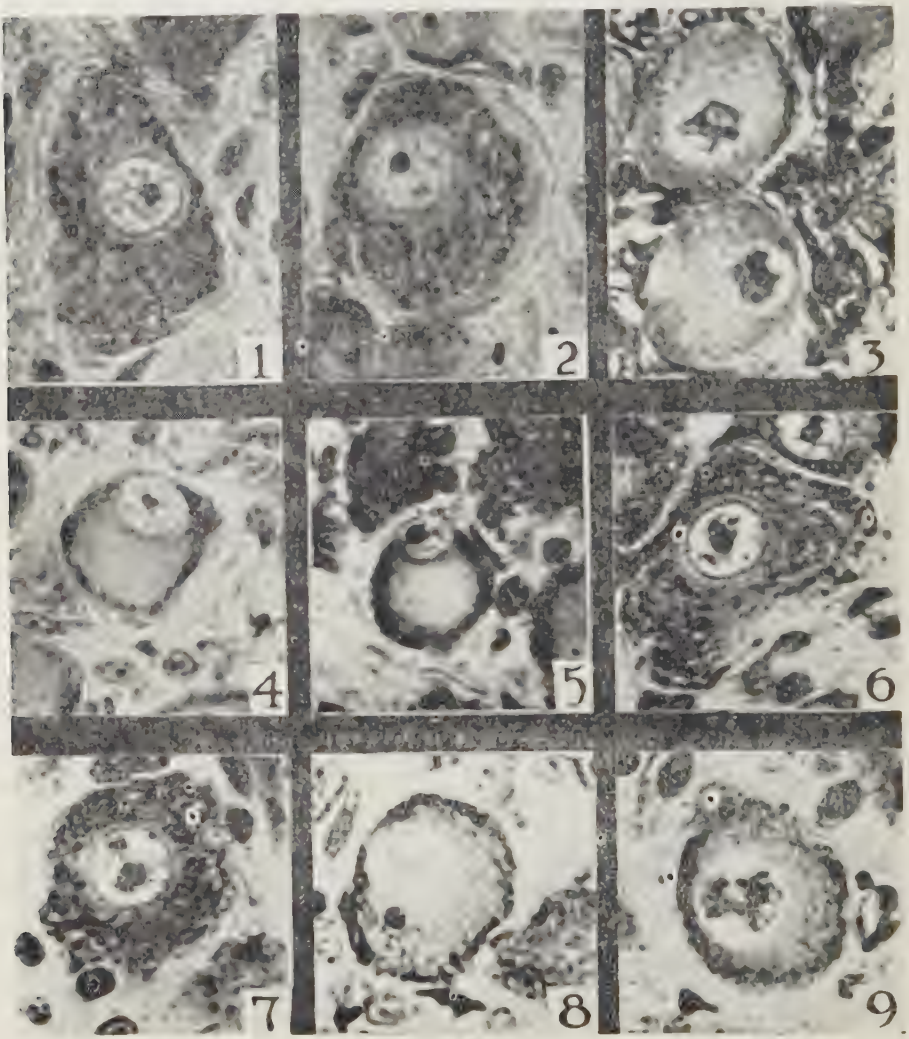
In observing the animals we have relied entirely on the classical physical signs of adrenal insufficiency. No blood or urine studies have been made because, for present purposes, we have been concerned solely with the autonomic ganglion cell picture and the degree of adrenal insufficiency is adequately indicated by physical signs alone. The animals show asthenia, anorexia, lowered body temperature, and circulatory disturbances corresponding to those outlined by other investigators.[1]

The study of ganglia for this report has been limited to the superior cervical and stellate. These ganglia were selected because of their easy accessibility and because of their distance from the operative field. However, the coeliac and the thoracic and abdominal chain ganglia show features in the experimental animals conforming to those described in the superior cervical and stellate. Each of the 18 cats from which ganglia were studied was suffering from adrenal insufficiency.

The ganglion cell changes in autonomic ganglia of the adrenalectomized animals may be mild or extensive, depending on the degree of insufficiency as indicated by the physical signs. But the degree of insufficiency is not a matter of time. It may become extreme in 3 days and some cats survive for 10 or 12 days. One cat which died on the fourth day showed more marked ganglion cell changes than another that died on the eleventh day after the final operation. The mild changes consist of swelling of cells and nuclei, chromatolysis, and disintegration of the Nissl substance which has a tendency to be more generally massed at the periphery of the cell than in the normal ganglion (Figs. 4 and 5). The more marked changes are pycnosis (Figs. 3 and 9) with occasionally extrusion of the nucleus from the cell (Fig. 5) and of the nucleolus from the nucleus (Fig. 8). The nuclear membrane may stain indistinctly or may appear fragmented. The cell bodies are often irregular and mark-

[1] Grollman, Arthur, *The Adrenals*, Williams and Wilkins Co., 1936, pp. 161-167.

edly shrunken and the Nissl substance either densely massed at the periphery, so that discrete granules can not be seen, or very finely granular and lightly stained. While the nuclei in autonomic ganglia have a tendency to be eccentric in position, that tendency is increased in the ganglia from the operated animals. The Nissl substance may



Figs. 1 and 2, normal sup. cerv., and figs. 6 and 7, normal stellate gang. cells. Fig. 3, pycnotic nuclei in sup. cerv. gang. cells 8 days after removal of second gland. Fig. 4, sup. cerv. gang. cell exhibiting early reaction. Fig. 5, sup. cerv. gang. cell, nucleus being extruded. Fig. 8, stell. gang. cell, nucleolus being extruded from nucleus. Fig. 9, pycnotic nucleus and peripheral Nissl substance. Fig. 3, iron hæmatoxylin; remainder, cresyl violet stain. × 500 before reduction.

normally be more abundant at the periphery of the ganglion cell, but it is often located centrally immediately surrounding the nucleus. Usually it is uniformly diffused throughout the cytoplasm. In the ganglia from cats suffering from adrenal insufficiency this substance is peripherally placed in the majority of cells (Figs. 4, 5, 8, and 9) but peripheral displacement alone does not indicate a degenerative change. The cell can only be considered as having an abnormal reaction in respect to Nissl substance when the latter loses its discrete granular character, a condition never encountered in normal ganglion cells in our studies. Vacuoles are frequently seen in the cytoplasm and when the nucleus is markedly shrunken the space it originally occupied remains as a vacuole between the shrunken nucleus and the cytoplasm (Figs. 3 and 9). The content of this space and other vacuoles does not stain with osmic acid nor with silver in our preparations. It is probably a fluid or semi-fluid substance.

The significance of the degenerative changes in autonomic ganglion cells in adrenal insufficiency is not entirely apparent from our experiments. Elliott[2] has offered experimental evidence to support his statement that animals deprived of their adrenal glands show a "nearly complete paralysis of vasomotor and cardio-accelerator nerves (all other nerves reacting in an approximately normal manner)." He found the vagus response to stimulus and pupillary reactions to be normal. Urechia and Mihaleschu[3] found similar changes in the nuclei of the *tuber cinereum*. They regard these nuclei as autonomic centers in the diencephalon. If the findings of Elliott are correct, the suggestion is offered that the ganglion cells degenerating in our animals are those concerned with vaso-constriction and cardiac acceleration, an explanation in accord with the fact that the great majority of the cells in these ganglia are undoubtedly vasomotor or cardio-accelerator in function. One might also suggest that the tuber nuclei showing degenerative changes in Urechia and Mihaleschu's experiments are probably vaso-constrictor and cardio-accelerator centers. However, a more extensive survey of the ganglia of the entire autonomic system and further study of autonomic centers will be necessary to warrant definite statements and further experiments directed to this end are in progress.

Ganglia other than the superior cervical and stellate do show degenerative changes in our series but the thoracic and abdominal chain ganglia appear to contain a greater proportion of normal cells than do the superior cervical and stellate. The coeliac is even more

2 Elliott, T. R., *J. Physiol.*, 1914, **49**, 38.

3 Urechia, G. I., and Mihaleschu, S., *Endocrinologie*, 1925, **3**, 296.

extensively involved than the latter ganglia, however. Attention has been called to degenerative changes in this ganglion in cases of Addison's disease.[4] Although Hollinshead[5] has shown that the nerve fibers to the adrenal medulla are preganglionic it seems likely that the cortex would receive some post-ganglionic fibers from the coeliac ganglion. Hence some of the changes in that ganglion may represent axonal reaction.

Summary. The cells of the autonomic ganglia of cats deprived of their adrenal glands for a sufficient time to bring about classical signs of adrenal insufficiency present definite degenerative changes. These changes vary in degree directly with the severity of the insufficiency as indicated by the physical signs. No definite statement can be made as to the significance of these findings in so far as our studies are concerned but the suggestion is offered that autonomic ganglion cell degeneration may explain some of the vasomotor disturbances in adrenal insufficiency. Neither is it possible to offer an explanation as to how the adrenal insufficiency affects the ganglion cells—whether as a part of a general metabolic disturbance or in some more direct, or even more complex, manner.

9194 P

Do Cysts of *Endamoeba histolytica* Formed in the Intestine Excyst Before Evacuation and Cause Internal Autoinfection?*

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The following study was designed to determine whether internal antoinfection from cysts of *Endamoeba histolytica* occurs. By internal autoinfection is meant the excystation of cysts formed in the intestine and their invasion of the tissues of the host without leaving the body. The term does not refer to "anus to mouth" transmission of cysts nor to reinvasion of the gut by the trophozoites.

Sellards and Theiler[1] claim to have infected 6 of 8 kittens by injecting material containing cysts only into the colon following

[4] McCrae, Thomas, *Osler's Principles and Practice of Medicine*, tenth edition, 1927, p. 882.

[5] Hollinshead, W. H., *J. Comp. Neurol.*, 1936, **64**, 464.

* Conducted in part as a project of the Amebiasis Unit of the National Institute of Health at Tulane University.

[1] Sellards, A. W., and Theiler, M., *Am. J. Trop. Med.*, 1924, **4**, 309.

laparotomy. In each case a silk ligature was tied around the large bowel and the inoculum injected above the point of ligation. Hoare[2] exhibited sections of the intestine of a kitten, presumably infected with *Endamoeba histolytica* by the rectal injection of cysts alone. According to Wenyon,[3] Drbohlav has stated that he was able to confirm these observations. However, these results are open to question, since the information for the most part is incomplete, there was a possibility of trophozoites being in the inoculum or the results were obtained under abnormal conditions.

Negative results were obtained by Izar,[4] who, in a single instance, inoculated one adult cat *per rectum* with a patient's stool containing cysts and presumably no trophozoites. No symptoms developed. Hegner, Johnson and Stabler[5] failed to produce infection in a brown howler monkey after injection of a human strain of cysts *per anum* into the colon.

In the present study 24 healthy young dogs, in addition to controls, were used. All animals were carefully examined before use to insure that none were naturally infected with amebæ. Precautions were taken to eliminate the possibility of the inoculum's reaching the mouth and thus causing infection by the normal route. Seven human strains of *Endamoeba histolytica*, known to be infective for dogs, were used to rule out individual differences of the strains. Both ripe and unripe cysts were present in the material used for inoculation and similar forms readily excysted when ingested by control animals. The inoculum in each case was exposed to low temperatures for a period sufficient to insure its freedom from viable trophozoites. The writer is indebted to Col. Charles F. Craig for complement fixation tests done on the animals before their use. All dogs gave negative reactions.

A number of attempts was made to determine, first, whether excystation occurred within reasonable time limits, and second, whether tissue invasion was produced. In the first series of 5 dogs, cysts were injected into the ileum following laparotomy, to determine whether excystation occurred in the ileum or large bowel without passage of the cysts through the stomach and small intestine. In all 5 animals no excystation occurred during periods ranging from 2 to 8 hours, which greatly exceed the normal time required for excystation when cysts are ingested. Dehydration was evident in some instances, but in others where such a condition did not exist, ex-

2 Hoare, C. A., *Trans. Roy. Soc. Trop. Med. and Hyg.*, 1925, **19**, 277.

3 Wenyon, C. M., *Protozoology*, 2 vols., 1926, 1563 pp.

4 Izar, G., *Beiheft z. Arch. f. Schiffs und Tropenhyg.*, 1914, **18**, 31.

5 Hegner, R., Johnson, C. M., and Stabler, R. M., *Am. J. Hyg.*, 1932, **15**, 394.

cystation likewise failed to occur. The same procedure, except that the inoculum was injected into the large bowel instead of the ileum, was repeated in 2 additional dogs. No excystation occurred.

To eliminate the unnatural conditions produced by laparotomy, cysts were injected into the cecum and colon *per anum*, with the use of the technic devised by Faust[6] for the transfer of the dysentery ameba from dog to dog. In these animals apparently viable cysts were found at autopsy from 3 to 12 hours after inoculation. It was evident that under these conditions neither excystation nor further development had taken place.

Since the dehydration was marked in some animals, even though large volumes of fluid had been given by mouth, it was found that agar aided in keeping the inoculum moist for several hours. Three dogs were given agar either by mouth or in the inoculum which was instilled into the colon *per anum*. The cysts likewise failed to excyst even when dehydration was slight and the medium of the inoculum in the intestine was moist or liquid.

Attempts to induce infection were made by producing stasis by various methods. Collodion plugs were inserted into the rectums of 4 dogs following inoculation with cysts by the anal route. These animals were autopsied from 2½ to 4 days later and only cysts were found, except that in one dog, which had freed itself and probably had ingested a portion of the voided inoculum, a single trophozoite was seen. Similarly, there was no evidence of excystation or tissue invasion in 3 dogs in which the anus was sutured to provide better retention of the inoculum.

The technic employed by Sellards and Theiler, who reported producing infection in cats by means of ligating the rectum following laparotomy and injecting cysts by needle into the lumen above the point of ligation, was repeated. One of 3 dogs inoculated in this manner became infected. In this animal, however, the large intestine was extremely dilated with fluid and gas, due to the stasis, and the mucosa was extremely inflamed. This condition was not quite so marked in the 2 negative animals in this series. It should be noted that this was the only infection produced in dogs by cysts which had not passed through the stomach and small intestine, and that this occurred under extremely abnormal conditions.

It is believed that these studies in the dog, which has been shown by Faust[7] to be a far more suitable experimental host for *Endamoeba histolytica* than the cat, indicate that internal autoinfection

[6] Faust, E. C., *Porto Rico J. Pub. Health and Trop. Med.*, 1931, **6**, 391.

[7] Faust, E. C., *Am. J. Trop. Med.*, 1932, **12**, 37.

by cysts does not normally occur, although it may be produced under extremely artificial conditions.

9195

Value of Mouse Brain Antigen for Diagnosis of Lymphogranuloma Inguinale.

EMMERICH VON HAAM AND RALPH HARTWELL.

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D'Aunoy and von Haam[1] reported the results of a series of Frei tests performed in 547 cases of lymphogranuloma inguinale and 1,132 negro hospital patients suffering from various other diseases. The diagnoses based upon the test proved correct in 90.9% of the cases, in 7.2% the patients gave doubtful reactions and in 1.9% apparently faulty reactions were obtained. A comparison of the various antigens used for the Frei test showed that antigen prepared from human glands and the brains of experimentally infected marmosets gave less doubtful reactions than the antigen prepared from diluted human pus or brain emulsions of infected mice.

Strauss and Howard[2] published the results of Frei tests on 14 persons which included 8 healthy controls, 5 definite cases of lymphogranuloma inguinale and one questionable case. They claimed that nearly half of the reactions to mouse brain antigen injected intradermally into normal subjects were indistinguishable from typical positive Frei reactions. These false reactions were ascribed by the authors to unknown changes which occur in infected mouse brain antigens within a few weeks after preparation. For this reason, the authors believe that Frei antigen made from mouse brain is unsuitable for the routine diagnosis of lymphogranuloma inguinale.

Grace and Suskind,[3] testing 95 specimens of their own lymphogranulomatous mouse brain antigens and 41 specimens of commercial mouse brain antigen in 50 cases of lymphogranuloma inguinale and 128 persons who served as control, denied the occurrence of any changes which would make the antigen unreliable for the performance of the Frei test, within a period of 2 years after preparation.

1 D'Aunoy, R., and von Haam, E., *Am. J. Clin. Path.*, 1936, **6**, 529.

2 Strauss, M. J., and Howard, M. E., *J. A. M. A.*, 1936, **106**, 517.

3 Grace, A. W., and Suskind, F. H., *J. A. M. A.*, 1936, **107**, 1359.

Because of the widespread interest that lymphogranuloma inguinale has recently aroused in the United States and the recognized value of the Frei test for the diagnosis of the disease, the question as to the value of the antigen used in the test is of extreme importance. In our own laboratory, mouse brain antigens have been prepared for the last 4 years at regular monthly intervals from various strains of lymphogranuloma inguinale virus and have produced very satisfactory results. Because of its quick method of preparation and standardization it has largely replaced antigens prepared from human material or from the brains of infected marmosets in our diagnostic and therapeutic work.

We have made a systematic study of the influence of age upon the specificity and the sensitivity of mouse brain antigen (M.B.A.). Small samples of each monthly-prepared portion of our antigen were preserved in a sealed vial either in the ice-box or in a desk drawer at room temperature and at the beginning of our experiments we had antigen available which was anywhere from one month to 14 months old. Antigen prepared from human material and control antigen prepared from normal mouse brain emulsions were likewise kept for over one year, under both conditions, and tested for diagnostic value. With this material, the sterility of which was frequently tested, Frei tests were performed in the usual manner and the reactions obtained were compared with tests performed on the same individuals with fresh human or mouse brain antigen. Five persons showing the clinical symptoms of acute lymphogranuloma inguinale and 68 healthy medical students who gave no allergic history were subjected to those tests, and the results are listed in Tables I, II, and III.

From the results shown in the tables we may conclude that neither the specificity nor the sensitivity of mouse brain antigen is greatly affected by ageing of the antigen up to 14 months. Out of a total of 177 reactions observed within a short time on 68 noninfected per-

TABLE I.
Frei Tests with M.B. Antigen Aged in the Ice-Box.
Medical Students.

Reaction	Age of M.B. Antigen in Months									Total No.
	14	12	10	8	7	5	4	2	1	
Negative										
up to 2 mm. diameter	37	5	6	4	4	8	5	2	6	77
Doubtful Negative										
3-4 mm. diam.	8	4	3	2	2	0	4	2	3	28
Weakly Positive										
5-6 diam.	2	0	0	0	0	0	0	4	0	6
Positive										
over 7 mm. diam.	0	0	0	0	0	0	0	1	0	1

TABLE II.
Frei Tests with M.B. Antigen Aged at Room Temperature.
Medical Students

Reaction	Age of M.B. Antigen in Months 14	12	10	9	8	7	5	1	Total No.
Negative up to 2 mm. diameter	6	2	15	7	5	5	6	8	54
Doubtful Negative 3-4 mm. diam.	0	3	3	2	1	1	0	1	11
Weakly Positive 5-6 mm. diam.	0	0	0	0	0	0	0	0	0
Positive over 7 mm. diam.	0	0	0	0	0	0	0	0	0

TABLE III.
Frei Tests with Old Antigen in Cases of Lymphogranuloma Inguinale.
Five Patients.

Type of Antigen	Age in Months	Reaction
Infected Mouse Brain	12, 10, 8, 7, 1	Strongly Positive
" Human Glands	12	Weakly "
Normal Mouse Brain	10, 1	Negative

sons, 74% gave reactions not larger than 2 mm. in diameter while in only 4% was the papule produced by the antigen larger than 4 mm. Control antigen prepared from the brain emulsions of normal mice and kept for 10 months under similar conditions likewise failed to produce papules larger than 4 mm. in diameter. A comparison of Tables I and II seems to indicate that ice-box temperature is not absolutely necessary to keep the antigens specific, provided their sterility is assured. From Table III we learn that old antigens maintain their reactivity for at least 14 months. Only the human antigen gave slightly weaker positive reactions after having been kept for one year in the ice-box. Although the number of patients with lymphogranuloma inguinale on whom the effectiveness of the old antigens was tested is not very large, we believe that the uniformity of the results in our cases is sufficient proof that the diagnostic value of mouse brain antigen is not altered by keeping the antigen in the ice-box or at room temperature for as long as one year.

The results of our investigation confirm the findings of Grace and Suskind. Without attempting to explain the contrary results of Strauss and Howard, we wish to stress some of the factors which seem of special importance in conducting the tests. The sterility of the antigen must be absolutely assured, the injection must be made in the superficial layers of the epithelium and the papules produced should not be larger than 10 mm. in diameter. Only persons in whom the presence of an allergic status has been excluded should

be used as normal control subjects. We agree with the New Haven workers that mouse brain emulsions will often produce strong reactions in allergic persons, but these reactions will be equally as severe following the injection of normal mouse brain emulsion as following the injection of specific mouse brain antigen. Therefore, we use normal mouse brain emulsions as control instead of sterile saline solutions, as first advocated by Frei.[4] A comparison of both reactions will then insure the correct diagnosis in persons with slight allergic reactions. This has also been advocated by Grace and Suskind.

Summary. On the basis of 217 Frei tests performed in persons suffering from acute lymphogranuloma inguinale and healthy medical students, with mouse brain antigen of varying age, we conclude that neither its specificity nor its sensitivity is altered by preservation in the ice-box or at room temperature, for as long as 14 months. In order to exclude false positive reactions in persons hypersensitive to mouse brain emulsions, the use of normal mouse brain emulsions as control is recommended.

9196 P

Effect of Posture on Cardiac Output.

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Most of the evidence obtained by various investigators employing different methods shows that cardiac output is decreased progressively in the sitting and standing as contrasted to the recumbent position. Recent investigations[1–5] in which the generally accepted more accurate acetylene method was used have given results in the same direction. Grollman,[6] however, using a nitrous oxide method which in his hands gave values agreeing with the acetylene method, found no change with posture. Beilschowsky,[7] using the acetylene method,

4 Frei, W., *Klin. Wchnschr.*, 1925, **4**, 2148.

1 Fisher, I. L., *Arbeitsphysiologie*, 1932-3, **6**, 111.

2 Bock, H., *Zeit. f. d. ges. exper. Med.*, 1934, **92**, 782.

3 Nylin, G., *Skand. Arch. f. Physiol.*, 1934, **69**, 237.

4 Schneider, E. C., and Crampton, C. B., *Am. J. Physiol.*, 1934, **110**, 14.

5 Scott, J. C., *Am. J. Physiol.*, 1936, **115**, 268.

6 Grollman, A., *Am. J. Physiol.*, 1928, **86**, 285.

7 Beilschowsky, P., *Klin. Woch.*, 1932, **2**, 1252.

also found no change. This discrepancy in the findings, augmented by Gladstone's criticism and modification of the acetylene method[8] prompted this reinvestigation of the problem.

Five experienced healthy adult males, ranging in age from 21 to 36 years, served as subjects for determinations made at intervals over periods of 4 to 6 months each. All determinations were made in the morning after the fasting subject arrived at the laboratory and had rested on a comfortable cot for at least 30 minutes. The recumbent determinations were made with the subject supine on the cot, the sitting in a straight-backed chair and the standing with the subject leaning lightly against the wall to prevent swaying. Changes in position were made with as little expenditure of energy as possible. Cardiac outputs were determined about 20 minutes after the assumption of the desired position.

All subjects show a smaller cardiac output in the standing as contrasted to the recumbent position (Table). This difference is further accentuated when only paired determinations (recumbent and standing done on the same day) are compared, the mean difference of 28 such determinations being 21%. The change is apparent regardless of whether the original Grollman[9] or the modified Gladstone method[8] is used. Values for the sitting approximate those for the standing position. A comparison of the results obtained by the 2 methods shows excellent agreement for outputs determined in the recumbent position but lower outputs due to higher A-V O_2 differences in the sitting and standing positions when the Grollman method is used.

Sampling at short intervals during the rebreathing (6 samples at intervals of 2 seconds or more) indicates an abrupt decrease in the rate of acetylene diffusion in the sitting and standing positions but

TABLE I.
Average of All Determinations, Gladstone Method.

	Recumbent				Standing			
Subject	No. of det'm.	O_2 cons. cc. per min.	C.O. liters per min.	Stroke vol., cc.	No. of det'm.	O_2 cons. cc. per min.	C.O. liters per min.	Stroke vol., cc.
F.	9	205.3	3.53	57.7	12	227.1	3.07	37.3
H.	9	197.4	5.31	91.9	16	223.5	3.91	41.3
M.	17	227.8	4.82	79.9	10	232.0	3.71	44.0
T.	12	236.8	5.65	109.1	12	289.8	5.16	61.9
M.	13	229.5	4.44	67.7	8	242.2	4.22	47.5
Average		219.4	4.75	81.3		243.0	4.01	48.0
% change						+10.8	—15.8	—40.9

[8] Gladstone, S. A., *Am. J. Physiol.*, 1935, **112**, 705.

[9] Grollman, A., *Am. J. Physiol.*, 1929, **88**, 432.

not in the recumbent position. This change occurs at 10.5 seconds (average 3 determinations) in the sitting and at 10.2 seconds (average 13 determinations) in the standing position and is obviously due to recirculation[8] although the factors involved in the more rapid recirculation in these positions have not yet been determined. These experiments account for the falsely high A-V O_2 differences and low outputs obtained with the Grollman procedure in the sitting and standing positions, since under these conditions the acetylene difference is erroneously low.

The adequacy of the short Gladstone rebreathing procedure for attaining homogeneity of gases in the lung-bag system prior to drawing the first sample has been substantiated. Two first samples were drawn simultaneously from the proximal and distal ends of the bag respectively. The resulting A-V O_2 differences calculated by pairing each of these first samples with the one second sample agreed closely. A-V O_2 differences calculated from any 2 samples in the 6-sample experiments also show good agreement from the end of the first expiration until recirculation takes place.

9197 P

Effect of Metabolites on Growth, and Differentiation in the Colon-Group.*

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The subdivision of the colon-group of bacteria into distinct subgroups is still a problem despite many studies. The genera *Escherichia* and *Aërobacter* can be differentiated from each other by nearly a dozen correlated characters. The genus *Aërobacter* can be further subdivided on correlated characters into at least 2 distinct subgroups represented by *Aërobacter aërogenes* and *Aërobacter cloacæ*. Aside from these divisions, however, considerable confusion still exists.

There are many strains whose characters are such that they cannot be allocated to either of the genera. In this paper these bacteria will be referred to collectively as "Intermediates".

* Aided by a grant from the Rockefeller Fluid Research Fund of Iowa State College.

TABLE I.

Relative Vigor of Growth of Colon-aërogenes Strains on "Staled" Agar Medium.

Test Organism		Escherichia				"Intermediates"				*Aër. aërogenes*				*Aër. cloacæ*			
		a	b	c	d	f	g	h	i	l	m	n	o	r	s	t	u
Culture Substrata		Escherichia "Staled" Agar.															
115	c	—	—	—	—	—	++	++	++	++	++	++	++	++	++	++	++
200	e	—	—	—	+	++	++	++	++	++	++	++	++	++	++	+	++
		Intermediate-group "Staled" Agar.															
148	i	++	++	++	++	—	+	—	—	++	++	+	++	++	+	+	++
277	j	++	++	++	++	—	+	—	—	++	++	++	++	++	+	—	++
		Aërobacter aërogenes "Staled" Agar.															
66A	l	++	++	++	++	+++	+++	+++	+++	—	+	—	—	++	++	+	+
244	o	+	++	++	++	+++	+++	+++	+++	—	+	—	—	++	++	++	++
		Aërobacter cloacæ "Staled" Agar.															
252	s	++	++	+++	++	+++	+++	+++	+++	++	+++	++	++	—	—	—	++
279i	t	++	++	+++	++	+++	+++	+++	+++	++	++	++	++	—	—	—	+

— No growth; + slight growth; ++ fair growth; +++ moderate growth.

Garré[1] and later Eijkman[2] showed that bacterial growth-products exhibit specific inhibitory effects. The purpose of this paper is to ascertain whether antagonism, or antibiosis, offers possibilities for differentiation of bacteria of the colon-group.

The method adopted was as follows: Broth containing 1.0% Bacto proteose-peptone and 0.1% K_2HPO_4 was inoculated with colon-organisms. These broth cultures were then incubated for 10 days at 37°C. An equal volume of a 3.0% agar gel was added to each of these 10-day broth cultures and plates were poured. This constituted what is known as a "staled" agar substratum.

These "staled" agar plates were then streaked with 24-hour broth cultures of the homologous organism and with 24-hour broth cultures of a number of other test-organisms employed in producing "staled" substrata. The cultures employed were 5 strains of the genus *Escherichia*, 6 "Intermediates", 6 *Aërobacter aërogenes*, and 6 *Aërobacter cloacæ*. Each of the 23 test-cultures was streaked on each of the 23 "staled" substrata, and the plates incubated for 48 hours at 37°C. Typical results are shown in Table I.

It is evident that the relative vigor of growth of an organism is distinctly better when streaked on a medium "staled" by an organism other than a member of its own genus or species than was the case with "staled" agar of the same genus or species. It is suggested that metabolic end-products (metabolites) are responsible for the effect observed.

Four 50 cc. samples of "staled" broth from 4 cultures were filtered through new, 155 mm., L5, Chamberland-Pasteur filter candles, agar gel added, and test cultures streaked as before. Distinct evidence of further growth was obtained, indicating that some of the inhibitory metabolites were adsorbed by the filters. Similarly, boiling a "staled" medium reduced its growth-inhibiting properties.

The results indicate the existence of adsorbable substances in cultures of *coli-aërogenes* organisms which seem to exhibit some evidence of specific, growth-inhibiting properties against homologous strains. It is suggested that these substances might assist in intergroup differentiation.

1 Garré, C., *Centbl. Bakt.*, 1887, **2**, 312.

2 Eijkman, C., *Centbl. Bakt.*, Abt. 1, 1904, **37**, 436.

9198 P

Effect of 2-4 Dinitrophenol on Oxygen Consumption of the Rabbit Lens.*

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Recent findings suggest that the therapeutic use of 2-4 dinitrophenol (DNP) in the treatment of obesity is followed in a small percentage of cases by the development of cataract.[1,2] Since the etiology of cataract is obscure and since metabolic disturbances in the lens may play a part,[3] an investigation of the action of this metabolic stimulant on the oxygen consumption of the lens was undertaken.

Young white rabbits about 1 kg. in weight were used throughout. These were killed by a blow on the back of the neck and the lenses rapidly removed, care being taken to avoid injury to the capsules. Excised lenses were placed at once in small (6 ccm.) vessels belonging to a set of 4 differential Barcroft microrespirometers. The suspension medium was mammalian Ringer's solution containing 0.2% glucose, buffered at pH 7.2 with M/150 phosphate. The vessels were then filled with oxygen and immersed in a water bath at 30° ± 0.01°C. The respirometer shaker was operated at a rate of 110 round trips per minute, with an excursion of 5 cm. Lenses were equilibrated for an hour under these conditions before measurement of respiration was commenced. These values of temperature and rate of shaking were maintained throughout. A total of 144 lenses was used in the 36 runs made.

Each lens served as its own control for a 60-minute period. DNP (as sodium dinitrophenoxide) was then added from the sidearms to the experimental vessels, Ringer's to the controls. In no case did such addition result in significant change of pH.

The oxygen consumption of the lens is relatively low. In cmm. oxygen (N.P.T.) per gm. wet weight per hour (Qo_2) the mean of 44 runs was 32.1 for the first hour of measurement. The range was 20.3 to 52.8, the standard deviation 7.4. There was a definite decrease in Qo_2 with increase in lens weight. Integral curves showing total oxygen consumption as a function of time are of the declining-

*Supported in part by Grant 400 of the Committee on Scientific Research of the American Medical Association.

1 Boardman, W. W., *J. Am. Med. Assn.*, 1935, **195**, 108.

2 Horner, W. D., *Arch. Ophthal.*, 1936, **16**, 447.

3 Bourne, M. C., *Physiol. Rev.*, 1937, **17**, 1.

rate type, although the decrease in rate is quite small (Curve I, Fig. 1, is typical).

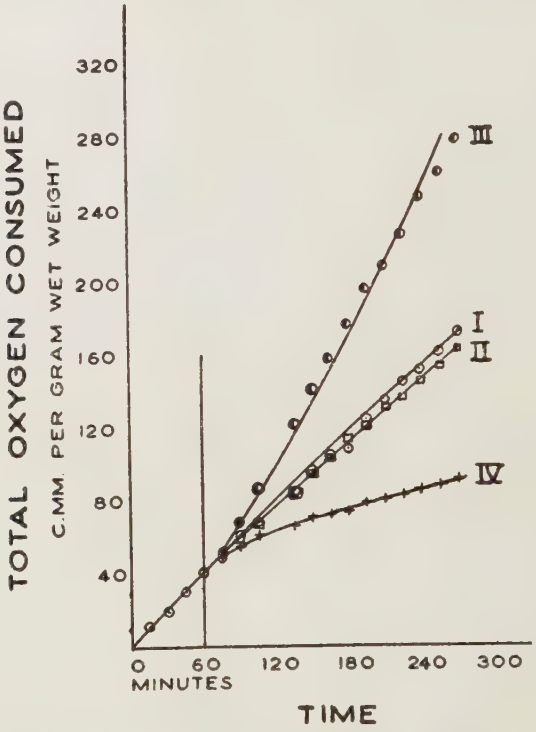

Fig. 1.

O_2 consumption of rabbit lens in Ringer's solution. Curves I and II represent respiration of lenses from one animal, III and IV from another. At 60 minutes DNP was added from the sidearms of vessels II, III and IV, Ringer's from the sidearm of I. The following were the concentrations of DNP in the several vessels. I, 0 (control); II, 0.005 mg. % (subliminal); III, 0.25 mg. % (stimulating); IV, 400.0 mg. % (inhibitory). On this scale curves I, II, III and IV are coincident for the first 60 minutes.

Twenty-five concentrations of DNP were employed, ranging from 0.005 to 400.0 mg. %. In general, concentrations above 5 mg. % were inhibitory. 0.05 to 1.25 mg. % stimulating, below 0.05 mg. % subliminal. The optimum concentration lay in the range 0.10 to 0.30 mg. %. Typical effects of subliminal, stimulating and inhibitory concentrations are shown in Fig. 1.

These studies are being continued and will be presented in detail in a subsequent paper.

9199 P

Observations on the Transmission of Anaphylactic Sensitivity in the Guinea Pig.

Milton B. Cohen and B. H. Woodruff.

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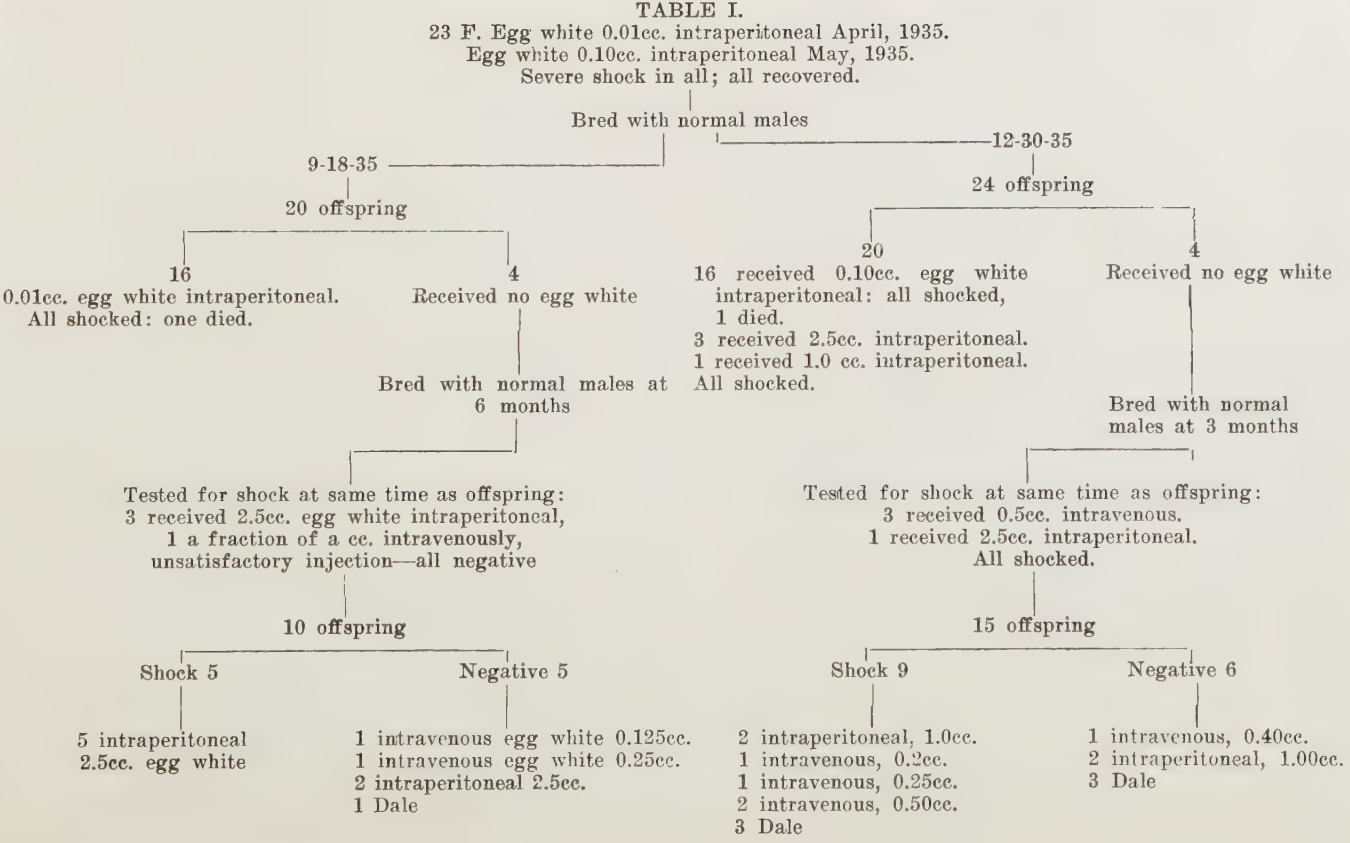
Ratner, Jackson and Gruehl,[1] in an exhaustive study of passive sensitization *in utero*, showed conclusively that when a female guinea pig was actively sensitized, and was bred with a normal male, all the offspring would be born sensitive as long as the mother remained sensitive. They used horse serum and demonstrated the presence of sensitivity by either the intravenous or intraperitoneal injection of the antigen or by means of the Schultz-Dale reaction. Passive sensitivity induced in this manner according to these authors "definitely persists for about 2½ months and may occasionally persist, to a moderate degree, up to 4 months. It will have worn off before the sensitized offspring matures and is capable of conception."

We have used the method referred to above to produce guinea pigs sensitive at birth to egg white. Chart 1 shows the details of our observations on the placental transmission of egg sensitivity from actively sensitized guinea pigs to their offspring and from these passively sensitized guinea pigs to over one-half of their litters. Instead of persisting for only 2½ months, as in Ratner, Jackson and Gruehl's series the passive sensitivity in our animals lasted long enough so that 4 females bred at 6 months of age had litters in which half could be demonstrated to be sensitive. All 4 mothers were tested after the birth of the offspring and none of these 4 suffered an anaphylactic shock. Similarly 4 passively sensitized females bred at 3 months of age produced 15 offspring, nine of which were born sensitive. All 4 mothers were tested after the birth of the offspring and each of these 4 animals suffered a definite anaphylactic shock with recovery when 181, 191, 193, and 203 days old.

These experiments demonstrated that passive anaphylactic sensitivity to egg white transmitted to a guinea pig *in utero*, lasts long enough to be transmitted through her placenta to at least half of her offspring if she is bred before 6 months of age.

1 Ratner, Bret, Jackson, Holmes C., and Gruehl, Helen Lee, *J. Immunol.*, 1927, **14**, 291.

TABLE I.


23 F. Egg white 0.01cc. intraperitoneal April, 1935.
Egg white 0.10cc. intraperitoneal May, 1935.
Severe shock in all; all recovered.
Bred with normal males
9-18-35
12-30-35
20 offspring
24 offspring
16
0.01cc. egg white intraperitoneal.
All shocked: one died.
4
Received no egg white
20
16 received 0.10cc. egg white intraperitoneal: all shocked, 1 died.
3 received 2.5cc. intraperitoneal.
1 received 1.0 cc. intraperitoneal.
All shocked.
4
Received no egg white
Bred with normal males at 6 months
Bred with normal males at 3 months
Tested for shock at same time as offspring:
3 received 2.5cc. egg white intraperitoneal,
1 a fraction of a cc. intravenously,
unsatisfactory injection—all negative
Tested for shock at same time as offspring:
3 received 0.5cc. intravenous.
1 received 2.5cc. intraperitoneal.
All shocked.
10 offspring
15 offspring
Shock 5
Negative 5
Shock 9
Negative 6
5 intraperitoneal
2.5cc. egg white
1 intravenous egg white 0.125cc.
1 intravenous egg white 0.25cc.
2 intraperitoneal 2.5cc.
1 Dale
2 intraperitoneal, 1.0cc.
1 intravenous, 0.2cc.
1 intravenous, 0.25cc.
2 intravenous, 0.50cc.
3 Dale
1 intravenous, 0.40cc.
2 intraperitoneal, 1.00cc.
3 Dale

9200 P

Observations on Human Subject Subsisting Six Months on a Diet Extremely Low in Fat.*

W. R. Brown, Arild E. Hansen, Irvine McQuarrie and G. O. Burr.

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Although it has been established that the animal organism is unable to subsist on a strictly low fat regimen,[1,2] relatively little is known concerning this type of diet as regards the human subject. The characteristic features found in rats reared on an extremely low fat diet as described by Burr and Burr[1] are scaliness of the feet and tail, early cessation of growth, hematuria and premature death. These animals also exhibit abnormal respiratory quotients. Burr and Burr[3] and Brown and Burr[4] have demonstrated the essential rôle of linolic acid as regards this deficiency syndrome. Von Gröer[5] following the work of Aran,[6] who had found that rats fed on fat-poor regimens failed to grow normally, maintained 2 infants on a diet practically free from fat for a period of several months. In the work of these investigators the vitamin factors were not controlled; however, it is interesting to note that one of these infants developed an eczematous eruption. Holt[7] recently found that one of 3 infants reared on a low fat diet developed eczema. No studies have been made on the adult human subject maintained on an extremely low fat diet for a prolonged period.

A normal adult was maintained on a diet consisting of carefully skimmed milk, sucrose, potato starch, baking powder, sodium chloride, orange juice, plus vitamin and mineral supplements, liquid petrolatum with anise oil and citric acid occasionally added for flavoring for a period of over 6 months. The total fat consumed per day was about 2 gm. and consisted chiefly of butter fat. Rats fed

* Aided by grants from the Medical Research Fund of the Graduate School of the University and the National Live Stock and Meat Board.

1 Burr, Geo. O., and Burr, M. M., *J. Biol. Chem.*, 1929, **82**, 345.

2 McAmis, A. J., Anderson, W. E., and Mendel, L. B., *J. Biol. Chem.*, 1929, **82**, 247.

3 Burr, Geo. O., and Burr, M. M., *J. Biol. Chem.*, 1930, **86**, 587.

4 Brown, Wm. R., and Burr, Geo. O. Presented at American Chemical Society meeting, section Biological Chemistry, at Kansas City, Missouri, April 16, 1936.

5 Von Gröer, F., *Biochem. Z.*, 1919, **97**, 311.

6 Aran, H., *Biochem. Z.*, 1918, **92**, 211.

7 Holt, L. E., Jr., Tidwell, H. C., Kirk, C. M., Cross, D. M., and Neale, S., *J. Pediat.*, 1935, **6**, 427.

on this diet developed the characteristic features of the fat deficiency syndrome of Burr and Burr[1] in the usual length of time. The following features were studied: Serum lipids using Bloor's[8] methods and the pyridine sulphate dibromide method[9] for iodine absorption capacity of the serum; arachidonic and linolic acid, using the same technique as described in the previous paper[10]; blood and urine analyses using the routine clinical laboratory procedures; blood pressure, mercury manometer; basal metabolic rate, indirect method; respiratory quotients, closed-chamber method; periodic physical examination by the same examining physician and careful notes on subjective symptoms by the experimental subject.

The following observations were made: *Serum lipids:* No change was found in the level of the cholesterol or total fatty acids, but the iodine number of the serum fatty acids fell from an average of 122 on normal diet to an average of 93 while on the fat-poor diet. This drop in the degree of unsaturation of the serum lipids confirms for the human subject the observation of several investigators[11, 12, 13] on various types of animals. *Arachidonic and linolic acids:* Values of 3.2% for arachidonic and 5.7% for linolic of the total fatty acids of the serum on normal diet compares with those found in pooled samples of blood from other normal subjects. There was a definite decrease in the content of both these acids on the low fat regimen, the values of 1.87% and 3.2% of the total fatty acids for arachidonic and linolic acids respectively being found. This decrease in the content of these highly unsaturated fatty acids tends to confirm the previous finding of a drop in the iodine number of serum fatty acids on the low fat regimen. *Blood and urine examinations:* The hemoglobin and red cell count remained essentially the same throughout the study. There was a definite tendency to a leucopenia, the leucocyte count at one time being only 2,000. The differential count remained unchanged. The fasting blood sugar level and glucose tolerance curve remained normal. The urine remained normal throughout the study, microscopic hematuria being especially watched for because of the finding of the bloody urine in rats

[8] Bloor, W. R., *J. Biol. Chem.*, 1928, **77**, 53.

[9] Yasuda, M., *J. Biol. Chem.*, 1931, **94**, 401.

[10] Brown, Wm. R., and Hansen, Arild E., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 113.

[11] Hansen, Arild E., and Burr, Geo. O., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1201.

[12] Williams, H. H., and Maynard, L. A., *J. Dairy Sc.*, 1934, **17**, 223.

[13] Hansen, Arild E., Wilson, W. R., and Williams, H. H., *J. Biol. Chem.*, 1936, **114**, 209.

on this diet. *Blood pressure:* The average blood pressure reading was about 10 mm. of mercury less after having been on the special diet for about 4 months than either before or after the return to normal diet. *Weight changes:* There was a gradual decrease in weight for the first 3 months on the diet. *Metabolism studies:* Basal rates by the indirect method were run by the hospital technician. Also, metabolic rates and respiratory quotients were found with a closed-chamber apparatus. With the former, rates of −11%, −11%, −2% were found, the highest value coming just before the low fat diet was abandoned. With the latter apparatus metabolic rates and R.Q.'s were found after starvation and during food utilization. Work with rats has shown that high metabolic rates, high dynamic action of food, and high respiratory quotients are to be expected when there is fat deficiency. The present studies showed no definite trend in metabolic rates (either before or after taking food), but higher respiratory quotients were encountered after the patient had been on the restricted regimen 6 months. These quotients were again lower 6 months after return to normal diet. The highest non-protein respiratory quotient (1.14) came in the sixth month on the diet. Eight months later the quotient under like conditions reached 1.01. *Objective clinical findings:* There was no significant change in the physical condition of the subject throughout the course of the study. Physical examinations made following resumption of normal diet were essentially negative. *Subjective findings:* The most noticeable feature was the marked absence of fatigue. Migraine attacks, which had been occurring at 7- to 10-day intervals, ceased after the subject had been on the diet for about 6 weeks.

9201 P

Experimentally Altering Galactin Content of the Rat Pituitary.

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In earlier communications we reported that the injection of the estrogens increased the galactin content of the male rat pituitary

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TABLE I.
Experimentally Altering Galactin Content of the Rat Pituitary.

No. of Animals	Sex	Physiological Condition	Aver. body wt. beginning of exper., gm.	Aver. body wt. when sacrificed, gm.	Aver. pituitary wt., mg.	Bird units per pituitary gland	Bird units per mg. pituitary tissue	Bird units per 100 gm. body wt.
10	F	Normal	42	123	5.1	1.4	.275	1.14
10	F	Ovariectomized (60 days)	42	149	7.3	0.86	.118	0.58
10	F	" (20 ")	150	174	7.9	2.73	.346	1.57
10	F	" + 500 I.U. theelin/day (20 days)	147	150	9.0	3.96	.440	2.64
5	F	Ovariectomized (18 days)	144	152	7.5	3.40	.453	2.24
5	F	" + 1000 I.U. Progynon-B/day (18 days)	145	138	16.3	5.87	.360	4.25
6	F	Ovariectomized + 200 I.U. Progynon-B/day (6-14 days)	149	144	14.4	10.00	.694	6.94
8	M	Normal		164	4.1	0.91	.222	0.55
8	M	Castrated for 35 days, then 25 I.U. theelin/day for 14 days 50 " " " " 18 " 100 " " " " 12 "	50	115	7.9	1.75	.222	1.52
9	M	Normal	232	211	6.2	1.05	.162	0.50
9	M	500 I.U. theelin/day for 18 days	232	199	8.9	1.69	.199	0.85

gland and that the galactin content of pituitary glands taken from male rats castrated for 60 days was not altered.

This report consists of further attempts experimentally to change the galactin content of the rat pituitary gland. Animals were paired upon the basis of body weight. At the close of the experimental period the control and experimental animals were sacrificed, their pituitary glands removed and assayed by injecting them over the crop glands of the same pigeons. The results are summarized in Table I.

Ovariectomy brings about a definite decrease in the galactin content of the pituitary gland. The injection of estrogenic hormones (theelin and Progynon-B†) into ovariectomized animals increased the galactin content of their pituitary glands over that of the ovariectomized, non-treated controls. It is interesting to note that the injection of 200 I.U. daily of Progynon-B brought about a greater increase in galactin content than did the daily injection of 1000 I.U. of Progynon-B. The mammary glands of the injected animals were quite extensive and well filled with secretion. Inasmuch as we have found that the pituitary glands of pregnant rats are somewhat lower in galactin than those of normal estrous cycle females and in light of the above observations it would seem that the estrogens do not cause a galactin storage or inhibition of galactin secretion during pregnancy. Theelin injections into castrated males increased the galactin content per pituitary gland but did not change the concentration of galactin per 1 mg. of fresh pituitary tissue, while theelin injections into normal males, 500 I.U. daily, increased the galactin content per pituitary gland as well as the concentration within the gland.

† The theelin and Progynon-B used in these experiments were kindly supplied by Dr. Oliver Kamm of Parke, Davis and Co., and Dr. E. Schwenk of the Schering Corporation, respectively.

9202 P

Growth of Mammary Gland of Hypophysectomized Guinea Pig.*

E. T. Gomez, C. W. Turner and R. P. Reece.

From the Department of Dairy Husbandry, Missouri Agricultural Experiment Station.

In a previous note,[1] it was reported that estrogens were ineffective in stimulating the growth of the mammary gland of hypophysectomized guinea pigs. Similar results have since been obtained when estrogen and progestin were injected simultaneously. These observations indicate either that the ovarian hormones stimulate the pituitary to the secretion of hormones which directly or indirectly cause the growth of the gland or require the supplementing effect of pituitary hormones to stimulate mammary gland growth. As the pituitary has been shown to stimulate the thyroid and adrenals, the products of these glands, thyroxine and adrenal cortical extracts, have been injected alone and in conjunction with estrogen into hypophysectomized male guinea pigs with negative results. The lactogenic as well as the thyrotropic and adrenotropic hormones of the pituitaries have also been tried without success.

Encouraging results have been obtained in one preliminary experiment which will be reported in the present communication. Three hypophysectomized male guinea pigs each received one male rat pituitary implant daily for 20 days. The donor rats had received 100 I.U. of estrogen (theelin in oil†) daily for 10 to 20 days before being sacrificed. The nipples of the guinea pigs showed gradual development and microscopic examination of the whole mounts of the mammary glands as well as sections revealed extensive alveolar development comparable with the extent of gland development induced by 20 daily injections of theelin into normal male guinea pigs.

A fourth hypophysectomized male received 20 daily implants of male rat pituitaries from animals which had not received theelin injections. The nipple and duct system remained rudimentary. Completeness of hypophysectomy was checked in each animal by section of the sella.

These observations appear to indicate that the route of action of the estrogens upon the mammary gland is by way of the pituitary.

* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 503.

1 Gomez, E. T., and Turner, C. W., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 404.

† Theelin was kindly supplied by Dr. Oliver Kamm of Parke, Davis and Co.

9203

Oestrogenic Treatment of Hypophysectomized Male Mice.*

E. T. Gomez, C. W. Turner, W. U. Gardner and R. T. Hill.

From the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, and the Department of Anatomy, Yale University School of Medicine.

With increasing numbers of investigations on the effect of oestrogenic treatment of hypophysectomized animals 2 schools of thought seem to have arisen. Individual studies on hypophysectomized rats and guinea pigs[1, 2, 3] seemed to show that in no case were the mammary glands able to respond to relatively large doses of female hormone. Other investigators[4–7] report that they could obtain normal mammary development even in the absence of the pituitary. One is at a loss to correlate these conflicting results, but it is hoped that this and future studies will help clarify the situation.

Hypophysectomy of the mouse is carried out in much the same fashion as in the rat or guinea pig. The hardiness of some strains of mice allows them to stand the operation very well, and postoperative recovery offers no significant difference from that described for the rat. In the male, the gonads and accessories begin regression at once, and approach a static condition about a month after operation. Regardless of when oestrogenic injections are started in hypophysectomized mice, they do not tolerate this treatment well, and a majority succumb within 48 hours. For this reason the number of animals herein described is not large, but analysis of the data is considered significant.

A total of 21 hypophysectomized mice were treated with various types of oestrogenic hormone. Of these animals 10 were later

* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 484. This work has also been supported in part by grants from the Fluid Research Funds of Yale University School of Medicine to Robert T. Hill.

1 Lyons, W. R., and Pencharz, R. I., Proc. Soc. Exp. Biol. and Med., 1936, **33**, 589.

2 Gomez, E. T., and Turner, C. W., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 320.

3 Reece, R. P., Turner, C. W., and Hill, R. T., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 204.

4 Nelson, W. O., Proc. Soc. Exp. Biol. and Med., 1935, **33**, 222.

5 Ruinen, F. H., *Acta Brevia Neerl.*, 1932, **2**, 161.

6 Freud, J., and De Jongh, S. E., *Acta Brevia Neerl.*, 1935, **5**, 47.

7 Asdell, S. A., and Seidenstein, H. R., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 931.

found to be completely hypophysectomized. In most of the animals the base of the skull was serially sectioned, and in some the remaining fragments were calculated on 3-dimension volumetric measurements.

Recently Hill and Gardner[8] have shown that small remaining fragments of anterior lobe will not maintain the gonads in either male or female hypophysectomized mice. Unpublished data (Hill and Gardner) show that in hypophysectomized male mice bearing ovarian grafts, the addition of a pituitary graft will allow growth of the mammary glands. The results now under discussion confirm these observations. Two animals, 4 and 44, retained 2 and 8% of the pituitary respectively, the fragments containing all 3 parts of the original gland. In the remaining incompletely hypophysectomized animals volume measurements were not made. The testes of these same 2 animals were very much regressed, as shown by tubule diameter and the condition of the accessory sex glands. It will also be noted that the adrenal cortex had undergone much involution as indicated by its reduced thickness. Gardner[9] has shown that female hormone will cause the replacement of the bony pubic symphysis by a ligament. In 3 of the cases reported in this paper, the same reaction had taken place, although the pituitary was completely absent.

Absolutely no mammary response was obtained by the treatment in those animals which were completely hypophysectomized. However, if even so small an amount as 2% of the pituitary remained intact, part of which was intermediate and posterior lobes, the mammary response was very good. The response obtained was essentially the same as that found in intact animals. Therefore in mice we may consider that some part of the anterior pituitary is essential to mammary response by theelin treatment. Our experience leads to the suggestion that in some types of experiments, especially those in testing oestrogenic hormone response on mammary glands, it is absolutely essential to know, by stained serial sections, just how much of the pituitary may remain intact. Small remaining fragments cannot be seen in fresh material, even with the aid of a low power dissecting microscope. It is certain that in dealing with anterior pituitary physiology, one must consider the fragment as well as the whole gland.

Summary. In completely hypophysectomized male mice, treated with female hormone, the mammary gland rudiments do not respond.

[8] Hill, R. T., and Gardner, W. U., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 78.

[9] Gardner, W. U., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 104.

TABLE I.

No.	Days injected	I.U. weekly	I.U. daily	I.U. Total	Pituitary Left %	Mammary response	Tubule diam. (microns)	Accessories	Thickness adrenal cortex (microns)	Symphysis
4	114	150 O.B.		2400	2	Good	90-100	Cast.		
16	8	150 "		150	0	None				
41	11	250 "		500	0	"	90-100	"	140-150	Loose
42	28	250 "		1000	0	"	70-80	"	140-150	
43	26	250 "		2000	0	"		"		
44	38	500 "		3000	8	Good	85-90	"		"
45	28	250 "		1000	0	None	70-80	"	145-155	"
2	30		50 P.B.	1500	0	"				
7	20		50 T.O.	1000	0	"				
8	20		50 "	1000	0	"				
13	20		50 O.B.	1000	0	"				
14	25		50 "	1250	0	"				
1	20		50 P.B.	1000	unmeasured fragments	Good				
3	60		50 "	3000	"	"				
5	20		50 "	1000	"	"				
6	20		50 "	1000	"	"				
9	20		50 "	1000	"	"				
10	20		50 T.A.	1000	"	"				
11	20		50 "	1000	"	"				
12	20		50 "	1000	"	"				
15	25		50 O.B.	1250	"	"				
	25	150 "		525	All	"	190-210	Normal	240-260	Loose 3-6 weeks

P.B. = Progynon-B.† T.O. = Theelin in oil.† T.A. = Theelin (aqueous).† O.B. = Oestroform-B.

The gonads, accessories and adrenal cortex undergo regression regardless of the female hormone present. The resorption of the pubic symphysis does not depend on the presence of anterior lobe material. A small fragment of prehypophysis enables the administered female hormone to induce growth of the mammary gland. Such a small pituitary fragment permits regression of the gonads, accessories and adrenal cortex. Caution must be urged in use of the term "physiologically hypophysectomized" animals.†

9204 P

Effects of Saponin and Digitonin on Lipase and Phosphatase Action.

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In the course of the investigation of the effects of various snake venoms on lipolytic action controls were run to determine whether any of the observed effects might be due to the decreased surface tension caused by the venoms. Two of the agents so employed were "purified" saponin, Merck, and Hoffman-LaRoche digitonin. The following communication is a report on the rather interesting observation that the former markedly inhibits the activity of pancreatic lipase but inhibits the lipase activity of blood only slightly, while digitonin in very low concentrations increases the lipolytic action of pancreatic lipase and has little or no effect on blood lipase action. That the activity in each case seems to be due to some factor apart from the effect on surface tension is indicated by the fact that both of these reagents are very powerful surface tension depressants. The inability of both to show the same marked effects on blood lipase seems to indicate that they are inactivated or removed by some constituent in blood serum. It is likely that the serum cholesterol combines with the saponin or the digitonin to form a very slightly soluble digitonide, thus removing the inhibiting or stimulating factor.

† Theelin was kindly supplied by Dr. Oliver Kamm of Parke, Davis and Co., and progynon-B by Dr. Schwenk of Schering Corp.

* Contribution No. 89 from the Department of Biology and Public Health, Massachusetts Institute of Technology, Cambridge, Mass.

Lipolytic activity was determined by the method of Sure, *et al.*,[1] with olive oil as the substrate and gum arabic, U.S.P., as the protective colloid. The enzymes employed were serum from fresh defibrinated horse blood, Eimer & Amend Lipase "pure", and Difco steapsin. The saponin solution contained 5 mg./cc.; lipase, 2.5 mg./cc.; steapsin, 2.5 mg./cc.; digitonin solution prepared according to the method of Schoenheimer and Sperry,[2] 1.4 mg./cc. The amount of lipolytic action was determined after 24 hours at 37° by titration with N/100 NaOH in alcoholic solution, using phenolphthalein as an indicator. The increase in acidity between 0 time and 24 hours is taken as a measure of activity. The results are tabulated as cc. of N/100 alkali used.

Tables I and II give the results of a few typical runs illustrating the relative effects of saponin inhibition and digitonin acceleration.

TABLE I.
Effects of Different Concentrations of Saponin on Blood and Pancreatic Lipase.

Saponin	Blood Lipase*	Eimer-Amend Lipase (cc. N/100 NaOH used)	Steapsin
0.0	4.2	12.45	1.80
0.5	4.1	8.65	0.35
1.0	4.0	7.95	0.10
2.0	4.1	6.85	0.10
3.0	3.9	6.25	0.10
4.0	4.0	5.55	0.05
5.0	3.4	4.50	0.10
	Final volume, 10 cc.		

*1.0 cc. blood serum used or 1.0 cc. 0.25% enzyme sol.

TABLE II.
Effects of Different Concentrations of Digitonin on Blood and Pancreatic Lipase.

Digitonin (mg.)	Blood Lipase (2 cc. used)	Eimer-Amend Lipase (cc. N/100 NaOH used)	Steapsin (at 20°)
0.0	10.6	8.1	0.6
0.7	10.6	5.8	0.6
1.4	10.5	11.0	1.0
2.8	10.6	14.5	1.7
4.2	10.4	15.1	1.9
	Final volume, 10 cc.		

From these results it can be concluded that Merck's saponin "purified" shows marked inhibiting powers toward pancreatic lipase, while digitonin shows marked accelerating action. The effects are not apparent with blood lipase except when very high concentrations are used, which seems to indicate that blood cholesterol precipitates

1 Sure, B., Kik, M. C., and Buchanan, K. S., *J. Biol. Chem.*, 1935, **108**, 27.
2 Schoenheimer, R., and Sperry, W. M., *J. Biol. Chem.*, 1934, **106**, 745.

the saponin, but after the cholesterol is exhausted inhibition is apparent.

Effect of Saponin on Phosphatases. The effects of saponin on blood phosphatase as well as on partially purified fecal, upper and lower intestinal phosphatase (kindly supplied by Dr. Harris of these laboratories) were determined. The method of determination was that suggested by Bodansky[3] and the inorganic phosphate liberated was estimated by the method of Fiske and Subbarrow.[4] As much as 15 mg. of saponin in 10 cc. of reacting mixture caused no inhibition of any of the phosphatase preparations used. It appears, therefore, that phosphatase activity is not appreciably influenced either by the action of saponin or the highly reduced surface tension of the reacting mixture solution.

9205

Cholesterol and Fatty Acids in Blood Plasma of Male and Female Rats.

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In the course of an investigation of cholesterol metabolism in the rat, a survey of the literature indicated a paucity of knowledge concerning the normal fluctuations of blood cholesterol in this species. The present communication deals with such values in normal adult albino rats of both sexes. Inasmuch as there is a large residue of free cholesterol in the erythrocytes, a circumstance essentially vitiating the determinations on whole blood, the necessity of using plasma or serum should be emphasized.

The rats used in this study were breeding stock and were of the Osborne and Mendel strain obtained from the Connecticut Agricultural Experiment Station. The females were not used until one month or more had elapsed after weaning of litters. All animals were fed a stock diet which includes a mixture of 97% G.L.F. calf meal[1] and 3% cod liver oil. During the lactation period the

[3] Bodansky, A., *J. Biol. Chem.*, 1933, **101**, 93.

[4] Fiske, C. H., and Subbarrow, Y., *J. Biol. Chem.*, 1925, **66**, 387.

* Sterling Fellow (1933-35).

† Commonwealth Fellow (1934-36).

[1] Maynard, L. A., *Science*, 1930, **71**, 192.

females received in addition, a paste food consisting of whole milk powder 25%, casein 25%, wheat germ 20%, and lard 30%. The food was removed approximately 15 hours before killing the animals.

Under ether anesthesia, the blood was drawn from the abdominal aorta into oxalated centrifuge tubes. All blood samples were secured within 5 minutes after the animal was anesthetized. Promptness in obtaining blood samples is necessary as is emphasized by the results of Mahler[2] who showed a definite rise in blood cholesterol, after 5 to 7 minutes, proportional to the duration of the anesthesia. With few exceptions 4 cc. or more of plasma were obtained from each animal; the plasma of 2 rats was combined in cases where it was impossible to obtain a volume of 4 cc. from an individual animal. The plasma was extracted with alcohol-ether (3:1) mixture and the precipitated protein washed with portions of boiling ethyl ether to insure complete extraction of the cholesterol. Total lipids were determined by Bloor's oxidative procedure.[3] The total and free cholesterol were determined by Okey's method of oxidizing the cholesterol digitonide as outlined by Boyd,[4] with an additional modification which will be described in a later publication.

In Table I are presented the concentration of the fatty acids in the plasma, and the percentage of free cholesterol in total cholesterol. The concentrations of the various cholesterol fractions are not reported in view of the work of Sperry and Schoenheimer,[5] which was recently published. According to these investigators oxalated blood plasma contains significantly smaller amounts of total and free cholesterol than either serum or heparinized plasma. However, they state that the total and free cholesterol are reduced in the same proportion. Therefore, it appears that the use of oxalated plasma has not affected the present results with respect to the relation of free and combined cholesterol.

Whereas in male rats, the free cholesterol in total cholesterol averaged 32%, with a range of 23 to 49%; in female rats the average value was 30% with a range of 20 to 38%. The average concentration of fatty acids in the plasma of the male rats was found to be 121 mg. per 100 cc. with an individual variation of 87 to 183 mg. per 100 cc.; and in the plasma of the female animals an average value of 150 mg., with a variation of 122 to 186 mg. per 100 cc.

2 Mahler, A., *J. Biol. Chem.*, 1926, **69**, 653.

3 Bloor, W. R., *J. Biol. Chem.*, 1928, **77**, 53.

4 Boyd, E. M., *J. Biol. Chem.*, 1933, **101**, 323.

5 Sperry, W. M., and Schoenheimer, R., *J. Biol. Chem.*, 1935, **110**, 655.

TABLE I.

The Concentration of Fatty Acids and the Percentage of Free in Total Cholesterol in the Blood Plasma of Male and Female Rats.

Males					Females				
Rat No.	Age (days)	Wt. (gm.)	Fatty Acids (gm./100 cc.)	Free in Total Cholesterol %	Rat No.	Age (days)	Wt. (gm.)	Fatty Acids (gm./100 cc.)	Free in Total Cholesterol %
1	268	336	183	23	**22**	**440**	332	176	24
2	180	514	150	49	23	439	251		
3	184	538	166	33	**24**	**373**	290	186	30
4	346	438	129	34	25	253	340	167	22
5	281	457	126	32	26	377	267	170	29
6	194	468	122	40	27	358	267	170	37
7	180	395	123	26	28	409	260		
8	181	487	125	35	29	415	330	156	37
9	268	371	112	25	30	330	341	154	24
10	359	580	117	34	31	176	358	142	23
11	175	485	123	34	32	369	306		
12	333	376	113	27	33	371	209	147	27
13	194	537	109	32	34	342	308	145	33
14	383	406	116	34	35	317	287		
15	352	480	103	33	36	395	299	138	27
16	311	443	98	30	37	597	261	136	25
17	550	360	102	33	38	379	254		
18	345	558	87	24	**39**	223	325	122	20
19	383	327	89	31	40	202	264		
20	381	313			41	395	237	128	34
21	182	435	—	35	42	485	257		
Average			121	32	43	281	246	136	37
					44	360	237		
					45	477	322	128	36
					46	489	230	—	33
					47	360	218		
					48	448	256	—	38
					49	395	276		
					Average			150	30

The relation of the cholesterol in the free state to that combined as ester appears to be the same in both the female and male animals, and is similar to that reported for human subjects,[6] although the concentration of total cholesterol in the plasma of rats is only approximately one-half that found in human subjects. The values for fatty acids indicate a higher concentration in the plasma of breeding female rats than in male animals.

9206 P

Phage-Specific Heat-Labile Factors in *B. dysenteriæ* Sonne.

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It was previously reported[1] that from a certain multivalent Shiga phage, most Sonne strains absorbed distinctly but only the rough fraction, *i. e.*, detectable only when a rough Shiga strain or a susceptible Sonne strain was employed in the test for residual phage. In these and earlier experiments[2] the bacteria were heated for 2 hours at 70°C.

Subsequent studies were then made with phages derived from chicken-stools and propagated with Sonne organisms. Although these phages gave equally strong reactions on all (15) Sonne cultures available, curiously enough none of our strains heated to 70°C. showed distinct absorptive effects. This result seemed to indicate the presence of heat-labile factors. So, we compared organisms heated at 56°C. and at 85°C. for one hour. Absorption was obtained only with some strains subjected to the milder degree of heat. The nature of absorbing and non-absorbing strains could not be correlated with the above-mentioned differences in ability to absorb the rough fraction of the Shiga phage, nor with the quality of smoothness or roughness, nor with the direct titer. Indeed, from one of the absorbing strains, smooth and rough variants were obtained and both exhibited thermal lability.

In view of the stability to formalin of heat-labile antigenic (flagellar antigens) and/or phage-specific factors (*V.* antigen of

6 Sperry, W. M., *J. Biol. Chem.*, 1936, **114**, 125.

* Aided by a grant from the Committee on Scientific Research of the American Medical Association.

1 Levine, Philip, and Beerman, P., *J. Immunol.*, 1936, **30**, 377.

2 Levine, Philip, and Frisch, A. W., *J. Inf. Dis.*, 1935, **57**, 104.

B. typhosus (Craigie)[3]), absorptions were made with formolized strains.† Thus treated, only the strains that possessed the heat-labile factor absorbed from the Sonne phage and they were just as effective as the bacteria heated at 56°C.

These observations on the Shiga and Sonne phages indicate that while thermostable Sonne factors are involved in reactions with the former phage, the latter (homologous) phage reveals heat-labile bacterial components.

The Sonne phage reacted intensely also on a small number of strains of *B. dysenteriæ* Flexner, but suitable absorptive tests failed to reveal the presence of corresponding fractions of phage. The absorbing surface of one sensitive Flexner strain studied in its relation to the Sonne phages, in contrast to the absorbing Sonne strains, was found to be thermostable.

Although both Shiga and Sonne phages are capable of lysing a number of Sonne strains, they differ strikingly in thermal stability. After the Sonne phage was heated at 65°C. for one-half hour, its titer for the homologous Sonne culture and the susceptible Flexner strain was reduced from 10^{-10} to 10^{-5}; after 4 hours at 65°C. the residual titer on both organisms was 10^{-1}, and no lysis whatever could be demonstrated after 5 hours' heating. In contrast, even after 5 hours at 65°C. the titer of the rough fraction of the Shiga phage on either Sonne or rough Shiga strains was hardly affected.

9207

Cobalt Content of Iron Compounds and Its Possible Relation to Treatment of Anemia.

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The physiological importance of cobalt has taken on greatly increased significance due to recent work in Australia and New Zealand demonstrating its value in the treatment of certain fatal diseases of sheep and cattle, which are characterized by progressive

[3] Craigie, J., and Brendon, K. F., *J. Path. and Bact.*, 1936, **43**, 233.

† The formolized bacteria were washed several times with distilled water to remove free formalin which inhibits phage action.

* Commonwealth Fund Service Fellow. On leave from the Department of Agriculture, Western Australia.

emaciation and anemia. Marston and Lines[1] reported success in curing "coast disease" of sheep in South Australia with doses of cobaltous nitrate supplying 1 mg. cobalt daily. Marked improvement in the live-weight, appetite and the anemic condition of affected animals followed the cobalt treatment. In the same year Underwood and Filmer,[2] working in Western Australia showed, by a fractionation method, that limonite ($Fe_2O_3.H_2O$) owed its potency in the cure of "enzootic marasmus" of cattle and sheep to its cobalt content. They had previously shown (Filmer and Underwood[3]) that its potency was not related to its iron content, since an iron-free fraction proved as effective as whole limonite. These workers showed further[2] that the disease in sheep could be cured with doses of cobalt chloride supplying as little as 0.1 mg. cobalt daily, and suggested that cobalt must be regarded as an essential element in animal nutrition. Marked increases in growth and improvement in the blood picture followed even this minute dosage with cobalt chloride. Filmer and Underwood[4] have fully confirmed these findings for sheep and have extended them to include affected cattle which respond to doses of cobalt chloride supplying only 0.3 to 1.0 mg. cobalt daily. They also report the interesting finding that the therapeutic action of sub-optimal doses of cobalt chloride was improved by the addition of traces of nickel.

Finally Askew and Dixon,[5] following on the above results, showed that the potency of the iron compounds used successfully in the treatment of "bush-sickness" of sheep in New Zealand was due to their cobalt content and reported success with minute doses of cobalt salts without added iron.

In view of these results it seemed likely that cobalt might be a very general contaminant of iron compounds, a factor of possible significance in the treatment of certain anemias where massive doses of such compounds are commonly used. Accordingly, a preliminary survey of a number of iron compounds, as found in this laboratory, was undertaken to ascertain their cobalt contents.

After separation of the iron as ferric chloride by continuous extraction of a strong hydrochloric acid solution of the iron compound with ethyl ether in a Kutcher-Steudel type of apparatus, cobalt was determined colorimetrically by the method of Kidson, Askew

1 Marston, H. R., aand Lines, E. W., *Aust. J. Council Sci. and Indust. Res.*, 1935, **8**, 111.

2 Underwood, E. J., and Filmer, J. F., *Aust. Vet. J.*, 1935, **11**, 84.

3 Filmer, J. F., and Underwood, E. J., *Aust. Vet. J.*, 1934, **10**, 83.

4 Filmer, J. F., and Underwood, E. J., *Aust. Vet. J.*, 1937. In press.

5 Askew, H. O., and Dixon, J. K., *N. Z. J. Sci. and Tech.*, 1936, **18**, 73.

and Dixon.[6] Duplicate determinations and recovery tests with added cobalt showed this method to be particularly adapted to the type of materials being analyzed. No losses of cobalt were experienced during the extraction of the iron.

The results are given in Table I. They are expressed as parts per million of whole material and as parts per million of iron.

TABLE I.
Cobalt Content of Iron Compounds.

Compound		Cobalt ppm. whole material	Cobalt ppm. of Fe
Ferric chloride	USP (i)	41	119
" "	USP (ii)	22	64
Iron wire	C.P.	40	40
Ferrous chloride	A.R.	13	47
" carbonate	C.P.	22	45
" ammonium sulfate	C.P.	2	14
" " "	A.R.	14	98
Iron and ammonium citrate	(i)	3	18
" " " "	(ii)	16	96
" " " "	(iii)	12	72
Ferric pyrophosphate	(i)	0.4	2.3
" "	(ii)	0.5	2.9

Though the number of materials analyzed is quite small, it is evident from the selection given that cobalt must be considered a very general contaminant of all common iron compounds. The amount present varies tremendously according to the source of the material and may apparently bear no relation either to the chemical nature of the compound or to its "grade" of purity in the ordinary chemical sense.

Whether this contamination with cobalt has any physiological significance where iron compounds are used therapeutically (other than in diseases of sheep and cattle previously mentioned) is not at present known. This can only be ascertained by proper experimentation. It is suggested, however, that the presence of these traces of cobalt may have a more significant effect than has been recognized previously. This possibility is enhanced not only by the extremely small amounts of cobalt found to be potent in animal metabolism[2, 4] and its known stimulative effect on the hematopoietic functions as evidenced by the production of polycythemia in the rat,[7, 8] but also,

[6] Kidson, E. B., Askew, H. O., and Dixon, J. K., *Ibid.*, 1936, **18**, 601.

[7] Waltner, A., and Waltner, K., *Klin. Woch.*, 1929, **8**, 313.

[8] Orten, J. N., Underhill, F. A., Mugrage, E. R., and Lewis, R. C., *J. Biol. Chem.*, 1932, **96**, 11.

in the hypochromic anemias of man, by the massive doses of iron compounds used.[9, 10]

The reasons underlying the necessity for such massive doses of iron have been the subject of speculation for some time. The possibility that contaminants play a significant part has been suggested by Sheldon and Ramage,[11] and others, while Heath, Strauss and Castle,[12] Fullerton,[13] and Witts[14] suggest, as a result of their experiments, that the primary factor governing the dosage of these compounds is their capacity to undergo absorption in the intestinal tract. This is said to be governed normally by their solubility and ability to yield free ferrous ions. Though the paramount importance of iron deficiency in the hypochromic anemias can hardly be denied, it is difficult to explain some of the phenomena associated with their treatment entirely on this basis. The "threshold phenomenon" mentioned by Witts[14] may be cited as an instance. In this connection the recent work of Brock and Hunter[15] is of decided interest and would seem to eliminate poor absorption as the explanation of the large doses required. These workers conducted iron balance experiments which showed that, though the iron in ferric ammonium citrate and Blaud's pill was poorly utilized to form hemoglobin, it was readily absorbed and was retained in the body in considerable amounts. It is evident that low utilization in hemoglobin formation does not necessarily imply low absorption in the intestinal tract. In commenting on these results Brock[16] suggests that the massive dosage may be necessary either to supply essential elements which may contaminate the iron compounds or to facilitate the absorption of other materials in the intestinal tract. This latter suggestion seems rather unlikely in view of the fact that large amounts of iron are known actually to decrease the absorption of some elements, *e. g.*, phosphorus.

Summary. A selection of common laboratory compounds of iron has been analyzed and found to contain significant, though extremely variable, quantities of cobalt. The possible significance of this finding in the treatment of certain types of anemia is discussed.

9 Sargent, W., *Lancet*, 1932, **222**, 230, 1322.

10 Witts, L. J., *Proc. Roy. Soc. Med.*, 1931, **24**, 543; 1933, **26**, 607.

11 Sheldon, J. H., and Ramage, H., *Quart. J. Med.*, 1932, **1**, 135.

12 Heath, C. W., Strauss, M. B., and Castle, W. B., *J. Clin. Invest.*, 1932, **11**, 1293.

13 Fullerton, H. W., *Edin. Med. J.*, 1934, **41**, 99.

14 Witts, L. J., *Lancet*, 1936, **230**, 1.

15 Brock, J. F., and Hunter, D., *Quart. J. Med.*, 1937, **6**, 5.

16 Brock, J. F., *Brit. Med. J.*, 1937, **13**, 314.

9208

Leucocytosis of Parturition.*

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The object of the present investigation was to ascertain if the increased numbers of leucocytes mobilized into the blood of pregnant women during labor were different chemically, and hence probably immunologically, from leucocytes already present. For this purpose, the lipid composition of the white blood cells was determined early in labor and again just before applying anesthesia prior to delivery. As previously demonstrated,[1] changes in lipid composition may be taken as evidence of changes in physiological activity of leucocytes. A demonstrable increase in the activity of blood leucocytes during labor would indicate that the leucocytosis of labor is a valuable defence mechanism against infection during delivery. No significant change in the lipid content of the white blood cells at this time would suggest that the leucocytosis is probably a coincidental phenomenon secondary to the muscular exercise of parturition. Evidence herein reported tended toward the latter view.

A study was made of 14 patients from the obstetrical service of the Kingston General Hospital. A sample of blood was taken as soon as labor had definitely begun and as early in labor as possible. A second sample of blood was taken just before anesthesia prior to delivery. Both samples were heparinized and a complete blood count, differential leucocyte count and the lipid content of the white blood cells determined on each. The method of separating and analyzing the white blood cells has been described.[2] The average length of time between the first and second samples of blood was 10 hours, the shortest interval was 3 hours and the longest was 25 hours. The patients were in all respects normal parturients, 8 being primiparous and 6 multiparous.

The average total leucocyte count in early labor was 7,100 cells per cmm., the lowest being 4,300 and the highest 10,000. The average total count at the end of labor and before anesthesia was 8,000, the lowest count being 5,700 and the highest 11,200. The average increase was 1,700 cells with a standard deviation of 1,060 cells. There occurred a leucocytosis in every instance, a leucocytosis which

* This work was aided financially by the Alice F. Richardson Fund of the Kingston General Hospital.

1 Boyd, E. M., *Surg. Gynecol. and Obstet.*, 1935, **60**, 205.

2 Boyd, E. M., and Stevenson, J. W., *J. Biol. Chem.*, 1937, **117**, 491.

was statistically significant and averaged approximately an increase of 24% in the number of white blood cells. There was no significant change in the differential count between early and late labor.

A statistical summary of the lipid values is presented in Table I. The average value in early and late labor, the average difference and the standard deviation of the average difference are shown. Standard deviation was calculated by means of a formula previously used.[2] The lipid content of the blood leucocytes was found lower, on the average, in these pregnant women than in non-pregnant women, confirming previous studies.[3]

TABLE I.
The Lipid Content of the White Blood Cells in Late Compared with Early Labor.

Value	Aver. Early Value mg. %	Aver. Late Value mg. %	Aver. Difference mg. %	Standard Deviation of Difference mg.
Total lipid	968	1057	+89	338
Neutral fat	140	170	+30	158
Total fatty acids	555	645	+90	217
" cholesterol	214	243	+29	57
Ester "	49	57	+ 8	33
Free "	165	186	+21	60
Phospholipid	583	669	+86	218

In the majority of instances there were small increases in the lipid values during labor. On the average all of the lipid values were slightly increased. But variations were so marked that it was impossible to conclude that parturition had any *significant* effect on the lipid content of the leucocytes. All of the average increases were within the range of the experimental error of the method.[4]

Since there is no marked change, if any, in the lipid composition of the blood leucocytes during parturition, there is probably no change in their individual physiological activity. Any increased resistance to infection conferred on the parturient woman by the leucocytosis of labor must be due to increased numbers of leucocytes or to increase in the opsonic power of the serum and not to more active leucocytes.

Summary. No significant change occurred in the lipid composition of the white blood cells of 14 patients during parturition, suggesting that the leucocytosis of parturition is not accompanied by the mobilization into the blood of a more active type of white blood cell.

3 Boyd, E. M., *Surg. Gynecol. and Obstet.*, 1934, **59**, 744.

4 Boyd, E. M., *J. Lab. and Clin. Med.*, 1936, **21**, 957.

9209

Alleged Influence of Glycerine and Diethylene Glycol upon the Irritating Qualities of Cigarette Smoke.

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Mulinos and Osborne[1] have reported that the addition of small amounts of glycerine (1 to 5%) to cigarette tobacco definitely increases the irritation by the smoke, while the addition of similar amounts of diethylene glycol markedly decreases this irritation. For criteria they used the degree and duration of edema and redness produced in the mucous membrane of the rabbit eye after instilling into the conjunctival sac water presumably saturated with cigarette smoke. This method is not quantitative. Flinn[2] has since reported that in patients suffering from irritation associated with the smoking of cigarettes (coughing, irritation of the tongue, congested pharynx and larynx) such conditions were cured or improved by smoking cigarettes containing diethylene glycol. Prompt return of the throat congestion occurred in 80% of the cases when glycerine was substituted for diethylene glycol, and in nearly all cases the tongue condition returned with this change. This method is also not quantitative.

It is known that within physiological limits irritation of the mucous membranes of the mouth gives rise to increased salivation. Tobacco smoke is an irritant known to stimulate salivary flow. If glycerine added to tobacco increases the irritating properties of the smoke, one would expect a greater flow of saliva from the smoking of tobacco treated with this hygroscopic agent, and if diethylene glycol markedly decreases the irritative properties, the reverse would be expected. With these considerations in mind, experiments were undertaken to measure the salivary responses of 28 persons (26 men, 2 women). This method of measuring the degree of irritation caused by smoke was employed because the buccal cavity is normally the place of entrance of tobacco smoke, and because it gives an objective quantitative measurement.

Thirteen one-hour tests were conducted upon each human subject; the first one acquainted the person with the procedure, but was not included in the final calculations. Each experiment consisted of 4 parts in this order: (1) 13 minutes of light reading while sitting in

1 Mulinos and Osborne, Proc. Soc. Exp. Biol. and Med., 1934, **32**, 241.

2 Flinn, *Laryngoscope*, 1935, **45**, 149.

a comfortable chair, at the end of which time any accumulation of saliva was removed from the mouth by a Dewitt dental saliva ejector and discarded; (2) 15 minutes of similar reading while saliva was being collected; (3) 15 minutes of puffing through a short resin or glass cigarette holder, each draw lasting 2 seconds and each exhalation about the same, a puff every half-minute, while in the interim the saliva was being collected continuously; (4) a final 15 minutes the same as under (2). The removal of saliva at the end of part (1) served also to moisten the inner walls of the collecting tube, insuring the smooth flow of saliva from the mouth to the 30 cc. graduated centrifuge tube. At all times during the collection the subject sat with head inclined forwards at an angle of about 45° so that the saliva would flow to the point of insertion of the ejector.

The apparatus used in these experiments of the salivary ejector connected with a short piece of pressure tubing with the measuring tube; suction was provided by a water suction pump, and a trap was inserted to catch any back-flow of water. The ejector and the cigarette holder were cleaned with boiling water between tests.

The tests upon each subject were run at the same time of day and under quiet conditions. It was agreed upon that there would be no smoking on the day of a test before the trial. There was no knowledge on the part of subject or experimenters as to the contents of the cigarettes. If during a test there occurred any mishaps, the results of that trial were discarded and another test substituted on another day. The 3 kinds of cigarettes used were made from the same tobacco, but one contained only tobacco, a second tobacco containing 2.61% of glycerine, and the third tobacco with 2.24% diethylene glycol, according to determinations made by the Miner Laboratories.

We classified the subjects into 3 groups based upon the amount of tobacco they were accustomed to smoke under ordinary circumstances. There were about equal numbers of non-smokers, of light and moderate, and of heavy smokers; an explanation of the latter 3 terms is given in Table I.

The average responses of the 28 subjects to the 3 kinds of cigarettes agreed very closely, so that there was not the slightest indication that one cigarette was more irritating than another. That the smoke did stimulate salivation is seen in the fact that smoking evoked an average of 50% greater flow of saliva than the puffing of air. Considering the averages for each of the 3 sub-groups, the variations are naturally greater, but in none are there significant differences in the response to the 3 tobaccos; very large variations in resting and in stimulated saliva are seen among individual subjects.

TABLE I.

Cubic Centimeters of Saliva Collected from Human Beings During Smoking Tests. Each Figure Represents the Average of 3 Fifteen-Minute Periods.

Subject	Pre-control	Air	Post-control	Pre-control	Glyc. tobacco	Post-control	Pre-control	Tobacco	Post-control	Pre-control	Diet.-Glyc. tobacco	Post-control
					Non-Smokers.							
WmL	2.5	4.0	3.5	3.1	14.0	3.3	2.7	13.6	4.2	3.9	15.1	5.4
YO	3.9	6.4	4.2	4.5	10.8	5.7	3.3	10.5	4.0	3.4	9.1	4.2
EB	4.0	2.8	3.5	2.9	2.4	2.4	2.7	3.6	2.3	3.1	3.4	3.4
JM	12.5	14.7	11.8	12.9	19.1	12.6	7.5	15.2	9.5	9.5	18.6	10.9
JD	8.7	10.3	10.0	7.9	15.5	7.9	7.5	14.6	7.7	7.4	13.2	7.7
HH	1.6	1.6	1.2	1.6	3.3	2.0	1.3	2.2	1.3	1.3	3.5	1.6
FB	18.8	21.3	19.5	15.5	21.7	18.6	16.4	20.6	20.0	18.8	24.1	23.1
FO*	11.1	12.7	10.1	8.8	15.1	9.0	9.3	18.6	10.2	9.6	17.1	9.7
SM	8.3	8.0	5.9	6.5	14.3	9.2	4.3	12.5	7.5	6.5	10.8	7.2
S*	0.5	3.6	0.9	1.0	8.1	1.1	0.0	9.0	1.2	0.7	10.0	1.1
SS	6.4	7.7	7.6	5.1	8.7	8.3	4.3	7.9	8.1	6.2	9.4	7.4
Sum	76.1	92.9	78.2	89.6	133.0	80.1	59.5	128.3	74.0	70.4	134.3	81.7
Aver.	6.9	8.4	7.1	6.3	12.1	7.4	5.4	11.7	6.7	6.4	12.2	7.4
			Light Smokers (1-5 cigarettes or equivalent daily), first 3 subjects. Moderate Smokers (6-11 cigarettes or equivalent daily), next 5 subjects.									
PB	2.0	3.5	3.4	2.5	14.3	4.9	1.4	13.4	3.6	2.1	13.2	3.7
HMc	8.0	12.6	7.7	8.7	22.2	11.5	9.9	29.2	12.9	10.2	26.6	14.6
RS	6.8	10.6	8.6	7.5	16.9	9.4	7.5	19.3	9.1	8.1	17.7	10.0
HS	14.5	15.5	13.0	15.3	28.7	12.6	13.1	28.7	13.3	13.8	27.7	11.9
NC	5.6	9.2	5.1	5.9	11.9	5.5	4.7	9.4	5.4	5.2	8.9	4.3
JP	10.0	12.9	9.6	9.0	17.5	12.0	14.0	20.8	16.2	10.5	19.7	13.4
NM	17.0	22.9	20.7	19.0	28.0	24.3	17.3	23.8	22.1	12.8	20.3	17.8
AM	1.8	4.2	3.0	2.9	11.7	4.4	2.6	13.5	4.1	1.8	12.1	3.8
Sum	65.6	91.4	71.1	70.8	151.2	84.6	70.5	158.1	86.7	64.5	146.2	79.5
Aver.	8.2	11.4	8.9	8.9	19.9	10.6	8.8	19.8	10.8	8.1	18.3	9.9

Heavy Smokers (12 or more cigarettes daily or equivalent)

DM	8.1	9.2	5.5	7.3	17.8	7.2	4.4	14.3	6.1	6.6	19.8	10.1
ST	4.7	6.6	4.2	4.2	7.0	6.3	6.0	8.9	4.3	5.9	8.2	4.5
AK	12.5	18.2	14.8	12.7	22.0	15.4	12.0	21.3	16.4	12.6	23.4	16.0
CS	5.4	7.8	5.4	2.4	9.3	5.8	4.7	9.8	5.4	5.2	8.9	4.3
WmS	4.7	5.4	4.1	2.5	5.0	1.7	5.2	6.5	3.6	1.7	2.0	0.6
EE	4.2	12.3	7.6	5.3	10.3	4.1	8.3	14.1	10.4	5.5	11.4	6.0
ChR	4.3	4.7	5.6	5.2	11.9	5.9	5.3	12.0	4.9	5.0	10.7	5.1
HB	1.0	1.7	0.8	0.6	8.1	1.6	0.4	7.6	1.3	0.7	7.5	1.7
LM	4.2	8.3	5.0	3.1	10.5	4.4	2.0	8.8	3.8	3.1	11.1	5.1
Sum	49.1	74.2	53.0	43.3	101.9	52.4	48.3	103.3	56.2	46.3	103.0	53.4
Aver.	5.5	8.2	5.9	4.8	11.3	5.8	5.4	11.5	6.2	5.1	11.4	5.9
Total Sum	190.8	258.5	202.3	183.7	386.1	217.1	178.3	389.7	216.9	181.2	383.5	214.6
Total Aver.	6.8	9.2	7.2	6.6	13.8	7.8	6.4	13.9	7.7	6.4	13.7	7.7

* Women.

In 8 of these people the glycerine-treated cigarettes evoked the greatest average response (stimulated saliva minus pre-control saliva), 12 times the cigarettes containing tobacco alone caused the most salivation, and in 8 persons the highest response followed the smoking of diethylene glycol-treated cigarettes. Here again there is no evidence that either of the treated cigarettes was more irritating than the other.

In order to determine to what extent these cigarettes are capable of classification as to irritating qualities in ordinary smoking we distributed among 29 students and research workers the 3 kinds of cigarettes previously described. Every subject received 3 envelopes containing 3 of the same kind of cigarette in each and was told to classify the smokes according to the 5 headings given in Table II.

TABLE II.

Classification of cigarettes based on the opinions of 29 subjects These persons tried each kind of cigarettes thrice but had no knowledge of the contents of the cigarettes. G—glycerine treated cigarettes. D—diethylene glycol treated cigarettes. T—cigarettes with tobacco only.

Name	Very Irritating	Irritating	Medium	Mild	Very Mild
JD		G T	G T	D D D	G T
AM		T T D	G G G T D D		
HS		G G T D D	G T T D		
YO		G G T D D D	G T T		
WmS		G G T	G T T D	D D	
DM		T D	G T D	G G T D	
JM		T T	G G T D D D	G	
MrsM		G T D	T T D D	G G	
NC	D D	D	T	G G T T	G
SS	G G	G T D D	T T D		
HMc		G T T D D	D	G G T	
HBr	T T D D	G G T D	G		
ChR		G G T	G T T D	D D	
SSm	G G G D	D D	T T T		
Fi		G	G G	D D D	T T T
MrsS	G G T T D D T	G D			
FS		G T	G G T D	T D D	
DeC	T	G T T	G D D	G D	
NM		G T D	G G T T D D		
EE		D D	G T T D	G G T	
WmL		G T T T	G G	D D	D
MissO	D D D	G T T T	G G		
Sch	G	T T T		G G D D D	
OL	D	D D		G T T	G G T
RW		G G T D G	D D	T T	
AM		G G G D D	T T T D		
LB		G	G G T D	T T D	D
CaH		G T D	G T	G T D	D
JSch		G G G D D	T T T D		
Summary:	%	%	%	%	%
Glyc.	8 (9)	32 (37)	27 (31)	16 (18)	4 (5)
Tob.	6 (7)	30 (34)	33 (38)	13 (15)	5 (6)
Dieth. glycol.	11 (13)	28 (32)	24 (28)	21 (24)	3 (4)

They were to be smoked in the manner customary to each individual and the subject wrote his opinion on the envelope immediately following the completion of the smoking. He or she did not look at the previous classification on the envelope before smoking, so that the opinion on every cigarette was quite uninfluenced. In these experiments also, neither subjects nor distributors knew which cigarette contained glycerine and which contained diethylene glycol; as in the preceding experiments all the results were at hand before the experimenter knew the composition of the cigarettes. The data are summarized in Table II.

Our data give no indication that cigarettes can be classified consistently as to the irritating quality of the smoke by supposedly normal humans, although Flinn's report suggests patients with various afflictions due to smoking are able to judge differences in cigarettes similar in nature to ours. In many cases the same kind of cigarette was at one time called mild and at a subsequent period pronounced irritating by the same person. We believe, therefore, that a method for determining the irritating properties of cigarettes which relies solely upon the opinions of ordinary smokers cannot be considered reliable.

The smoke of these 3 types of cigarettes increases the acidity of water to an equal extent, as determined by exact pH tests.

9210

Response of Anterior Pituitary of Immature Castrated Rat to Testosterone and Related Compounds.*

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It has been reported that injections of extracts containing the male sex hormone prevented the post-castration increase in the size and number of the basophilic cells in the anterior hypophysis of the rat.[1, 2] In later studies, Nelson and Gallagher[3] studied the action

* These studies have been aided by grants made to Vanderbilt University Medical School by the Division of Medical Sciences of the Rockefeller Foundation and the Grants-in-Aid Committee of the National Research Council.

1 Reese, J. D., and McQueen-Williams, M., *Am. J. Physiol.*, 1932, **101**, 239.

2 Nelson, W. O., and Gallagher, T. F., *Anat. Rec.*, 1935, **64**, 129.

3 Nelson, W. O., and Gallagher, T. F., *Science*, 1936, **84**, 230.

of synthetic male factors, androsterone and related compounds, in castrated female rats. Such injections prevented castration changes in the pituitary; they also state that these injections induced the same type of degranulation as had been obtained by the injection of oestrin. Below are presented studies on the action of testosterone and its related compounds, testosterone-acetate and testosterone-propionate on the anterior pituitary of castrated immature rats.

Twenty immature rats, mostly males, castrated immediately before injections were begun, received daily injections of 500 gamma of either testosterone,† testosterone-propionate, or testosterone-acetate for 10 days; the animals were sacrificed 24 hours following the final injection. Twenty-three littermates castrated and killed at

TABLE I.

Frequency Distribution Table Showing Level of Cell Types in Injected and Non-injected Castrated Immature Rats.

Intervals in %	Frequency Distribution Control	Experimental	Mean % Control	Experimental
Eosinophiles:			%	%
30.0-34.9	9	6		
35.0-39.9	11	10	M = 36.0	M = 37.0
40.0-44.9	3	4		
Basophiles: (Granular)				
0.0- 0.9		6		
1.0- 1.9		7		
2.0- 2.9		2		
3.0- 4.9		5		
5.0- 6.9	2		M = 11.0	M = 1.7
7.0- 8.9	5			
9.0-10.9	5			
11.0-12.9	5			
13.0-14.9	3			
15.0-16.9	3			
Basophiles: (Non-gran.)				
0.0- 1.9	3			
2.0- 3.9	8	1		
4.0- 5.9	10	7	M = 3.7	M = 6.4
6.0- 7.9	2	9		
8.0- 9.9		3		
Chromophobes:				
35.0-39.9	2			
40.0-44.9	3			
45.0-49.9	8	4	M = 49.3	M = 54.9
50.0-54.9	4	7		
55.0-59.9	6	5		
60.0-64.9		4		
Mean Pituitary Wt.	5.7	4.9		

† The testosterone and testosterone propionate were furnished by the Schering Corporation through the courtesy of Dr. Erwin Schwenk. Testosterone-acetate was furnished by the Ciba Corporation through the courtesy of Mr. Robert Mautner.

the same time as the injected rats were used as controls. The prostate and seminal vesicles were weighed together. Their mean combined weight in the injected rats was 663 mg., the range from 279 to 1,010 mg. The mean combined weight of these organs in the control rats was 40 mg. The mean pituitary weight of the castrated control rats was slightly greater than that of the injected rats (Table I).

The pituitaries of all rats were studied histologically; cell counts were made on all sections studied. In the castrated control rats there was a marked increase in the size and the number of the basophilic cells (Table I). A majority of these cells were well packed with granules; the negative image of the Golgi apparatus was quite prominent, usually being more or less circular in form and situated between the nucleus and the cell membrane. A lesser number of these large basophiles appeared degranulated; in some of these cells small yellow-orange bodies, thought to be mitochondria, were observed in the blue cytoplasm. The negative image of the Golgi apparatus was large and prominent. The mean level of the eosinophiles was 36%; the range of variation was slight (Table I). The mean level of the chromophobes was 49.3% (Table I). Mitoses were found in the chromophobes and occasionally in the eosinophiles; counts revealed there was a mean of 5 per section in the chromophobes and 1 per section in the eosinophiles.

Injection of either testosterone, testosterone-acetate or testosterone-propionate suppressed the increase in the number and size of the basophiles which occurred after castration in the control rats. Furthermore, there was evident a marked degranulation in the basophiles in the injected rats; the mean level of granular basophiles was reduced to 1.7%. Degranulated basophiles were quite abundant; most of these were moderate in size. In some, small clumps of basophilic granules were found. Small yellowish-orange bodies, thought to be mitochondria, were often observed in the blue cytoplasm. The negative image of the Golgi apparatus was usually observed in these cells. Testosterone-propionate was most effective in preventing castration changes and inducing basophilic degranulation.

We were unable to detect any constant changes in the eosinophiles of the injected rats; the levels of these cells in the control and experimental groups were almost identical. In some few instances in the injected group a few eosinophiles were observed which appeared to present a reduction of granules but this finding was quite inconstant. It is possible that if we had injected larger amounts, changes might have been induced in the eosinophiles.

The percentages of the chromophobes in the injected group were slightly higher than in the castrate-control group. This was due, however, to the fact that the relative levels of the basophiles were much lower in the injected rat. As a whole, the chromophobes in the injected rats were normal in appearance; infrequently large, swollen cells with a hypertrophied negative image of the Golgi apparatus were observed. Counts of the mitotic figures revealed that they occurred in practically the same numbers as in the castrated control rats.

These studies indicate that the action of testosterone and related compounds on the anterior lobe differs from that of oestrogenic substances. The latter induce a weight increase in the pituitary, degranulation of many and often all of the basophiles and degranulation of a lesser number of eosinophiles. The chromophilic cells are reduced in percentage; the chromophobes are increased.[4] On the other hand, we have been able to detect only degranulation of the basophiles after injection of the male hormone; there is the possibility that injection of large amounts would result in changes in the anterior lobe similar to those induced by oestrin. However, other evidence indicates that the pituitary response to oestrin and to testosterone differs. It has been reported that injection of testosterone-acetate simultaneously with oestrin in either normal or castrated female rats partially suppressed the capacity of oestrin to induce pituitary hypertrophy and degranulation of the chromophilic cells.[5] These findings seem of considerable significance in view of the report[6] that injection of large amounts of the anterior pituitary-like factor (A.P.L.), simultaneously with oestrin, into either normal male or female rats partially suppressed the action of oestrin on the anterior lobe in the same manner as did the male hormone. However, A.P.L., injected simultaneously with oestrin in castrated female rats, failed to modify the response of the anterior lobe to oestrin. It is suggested that the ovary, under the stimulating influence of A.P.L., produced some factor that exerted the same suppressing effect on the action of oestrin on the anterior pituitary as did the male hormone. It is impossible to say whether this substance was actually the male hormone. However, this possibility is rendered more tenable by the finding that transplanted ovaries in castrated male mice were able to maintain the accessory reproductive organs.[7]

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4 Wolfe, J. M., and Chadwick, C. S., *Endocrinology*, 1936, **20**, 503.

5 Wolfe, J. M., and Hamilton, J. B., *Anat. Rec.*, 1937, **67**, 55 (Suppl.).

6 Wolfe, J. M., *Anat. Rec.* (in press).

7 Hill, R. T., and Gardner, W. U., *Anat. Rec.*, 1936, **64**, 21.

9211

Effect of Splenectomy on the Therapeutic Action of *p*-Aminobenzenesulfonamide on Mice Infected with Hemolytic Streptococcus.

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Sufficient experimental and clinical data have been reported by various authors, particularly by Colebrook and Kenny,[1, 2] to establish beyond all doubt the therapeutic effectiveness of *p*-aminobenzenesulfonamide and related diazo-sulfonamide compounds (Prontosil and Prontosil, soluble) in hemolytic streptococcal infections.

Ever since the discovery of Prontosil by Domagk,[3] the mechanism of the action of this group of chemicals has been a puzzling question. No evidence of bacteriostatic or bactericidal action of Prontosil had been proved until Colebrook, Buttle and O'Meara[4] demonstrated such action with *p*-aminobenzenesulfonamide against small numbers of hemolytic streptococci in culture medium and in blood. These investigators found Prontosil to be inactive as previously reported, but were able to demonstrate inhibitory effects when Prontosil was reduced with magnesium powder under partial vacuum. Long and Bliss[5] were also able to "activate" Prontosil by reduction with sodium formaldehydesulfoxalate.

As has been pointed out by Colebrook and coworkers,[4] and by Long and Bliss,[5] there is a wide discrepancy between the relatively small bacteriostatic and bactericidal action of the drugs *in vitro* and the excellent *in vivo* results. This discrepancy suggests that the bacteriostatic action may not be an essential factor in the therapeutic results obtained.

Colebrook, *et al.*,[4] suggest that the bactericidal action of the tissues of the whole animal may be important; whereas Long and Bliss[5] believe the phagocytic activity of the polymorphonuclear leukocytes and monocytes plays a paramount rôle in controlling infections caused by the beta-hemolytic streptococcus.

Our own investigations[6] on over 200 mice infected with 2 differ-

1 Colebrook, L., and Kenny, M., *Lancet*, 1936, **1**, 1297.

2 Colebrook, L., and Kenny, M., *Lancet*, 1936, **2**, 1319.

3 Domagk, G., *Deutsche Med. Wchnschr.*, 1935, **61**, 829.

4 Colebrook, L., Buttle, G. A. H., and O'Meara, R. A. Q., *Lancet*, 1936, **2**, 1323.

5 Long, P. H., and Bliss, E. A., *J. A. M. A.*, 1937, **108**, 32.

ent strains of hemolytic streptococci have shown no greater indication of phagocytosis in treated animals (as demonstrated in smears of peritoneal exudate and heart blood, as well as in sections of the livers and the spleens) than in the untreated controls. We are, therefore, not inclined to accept the hypothesis of Long and Bliss.

Various authors have theorized upon the possible rôle of the reticulo-endothelial system, which, when presumably stimulated by the sulfonamide compounds, supposedly combats streptococcal infections. No experimental evidence has been offered in support or refutation of this concept, except by Bosse,[7] who claims to have demonstrated the lack of protective action of Prontosil against hemolytic streptococcal infections in splenectomized mice. Unfortunately, no details regarding the number of mice employed, or the manner in which the experiment was controlled were given.

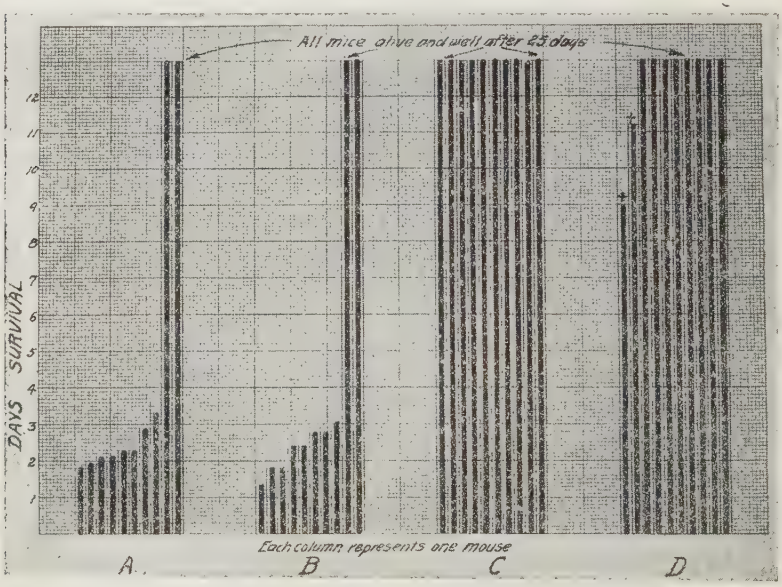

FIG. 1.

Graph of the survival time of mice infected intraperitoneally with the "Stoddard" hemolytic streptococcus (0.5×10^{-9} cc. of a 24-hour broth culture).

A. Normal untreated controls.

B. Splenectomized untreated controls.

C. Normal mice treated with 25 mg. *p*-aminobenzenesulfonamide by mouth immediately after infection and every 24 hours for 9 consecutive days.

D. Splenectomized mice treated in a manner identical to those in C. The columns marked + represent mice which at autopsy showed no streptococci in either the peritoneal cavity or the heart blood.

[6] Mellon, R. R., Gross, Paul, and Cooper, F. B., *J. A. M. A.* In press.

[7] Bosse, O. A., *Fortschritte D. Therap.*, 1936, **9**, 540.

The sections of livers and spleens from our several series[6] of mice infected with hemolytic streptococci and treated with Prontosil or *p*-aminobenzenesulfonamide, as well as sections from the untreated controls, failed consistently to show any morphologic evidence of activity on the part of the reticulo-endothelial system. This contradictory finding suggested an investigation of Bosse's claim.

The culture used was the "Stoddard" strain of hemolytic streptococcus in its mucoid phase. Preliminary titrations of a 24-hour broth culture on both normal and splenectomized mice showed no significant difference in their susceptibility and placed the lethal dose at 0.5×10^{-9} cc.

Twenty splenectomized mice which had been allowed to recover from the effects of the operation for a period of 3 to 4 weeks, and 20 normal mice were inoculated intraperitoneally with 0.5×10^{-9} cc. of a 24-hour broth culture. Ten of the normal, and 10 of the splenectomized mice were treated with 25 mg. of *p*-aminobenzenesulfonamide by mouth immediately after infection, and every 24 hours thereafter for 9 consecutive days, after which the treatment was discontinued. The other infected animals, 10 normal, and 10 splenectomized, were not treated, and served as controls.

The normal, as well as the splenectomized controls, showed an 80% mortality rate in 3 days, 2 mice surviving in each group. All mice in the normal treated group survived, whereas there were 2 deaths in the splenectomized treated group—one on the tenth, and one on the twelfth day. At autopsy, these 2 animals showed no streptococci in either the peritoneum or the heart blood. The results are shown graphically in Fig. 1.

Conclusions. 1. *P*-aminobenzenesulfonamide is capable of protecting splenectomized mice against fatal doses of highly virulent hemolytic streptococci. 2. The degree of protection observed in splenectomized mice was identical with that obtained with normal animals.

9212

Intra-ocular Heterotransplantation of Gonads and Sex Accessories from the Albino Mouse to the Albino Rat.

Clarence D. Turner. (Introduced by C. W. Turner.)

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Earlier observations by the author[1] indicated that the anterior chamber of the eye was an exceptionally favorable site for the incorporation and persistence of testicular homografts. Grafts of this type proliferated mature germ cells and produced detectable amounts of male hormone for periods as long as 15 months. Browman[2] found that the testes of albino mice transplanted subcutaneously, intramuscularly and intraperitoneally into albino rats sometimes persisted for several months but were not demonstrably functional. Since homotransplanted tissues have been reported to persist in the aqueous humor of the anterior chamber of the eye and to retain their histological differentiations for relatively long periods without vascularization from the iris,[3] it became of interest to determine to what extent heterotransplanted organs would persist and function following insertion into the anterior chamber.

Fifty-six testes and 22 ovaries from prepubertal mice ranging from one to 20 days of age were transplanted bilaterally to the eyes of castrated male and female rats varying from 22 to 264 days of age. The heterotransplants were observed through the corneas, and attempts were made to recover the transplants at intervals from one day to 5 months. Within the first day after insertion of the gonadal transplants violent leucocytic reactions were observed in the eyes. The anterior chambers became hemorrhagic and inflamed and by the third day considerable exudation occurred through the unhealed incisions in the corneas. These incisions usually did not heal thoroughly until about 20 days, or until the heterotransplant had been eliminated. The anterior chambers invariably remained cloudy for 15 to 30 days and during this period the presence of donor tissues could not be detected except by sectioning. Only 4 testicular (7.14%) and 6 ovarian (27.27%) transplants were recovered subsequent to persistence for 20 days. The recovered transplants were found to be fibrotic or calcified nodules and contained no recognizable traces of the donor tissues. Since Browman

[1] Turner, C. D., *Anat. Rec.*, 1936, **67**, 112.

[2] Browman, L. G., 1935, Thesis, University of Chicago.

[3] Haterius. H. O., Schweizer, M., and Charipper, H. A., *Endocrinology*, 1935, **19**, 673.

recovered 57% of his testicular heterografts subsequent to persistence for 6 to 45 days in subcutaneous, intramuscular and intraperitoneal positions, it appears that the transplants were more rapidly eliminated from the anterior chambers than from other sites. In no case did the heterotransplanted gonads in the anterior chambers maintain secretory processes in the male accessory glands or prevent the loss of oestrus cycles in the female hosts. Castration changes in the pars distalis of the hypophysis were not retarded by testicular heterotransplants.

Sixteen vesicular glands and 12 ventral prostatic lobes from 30-day-old mice were transplanted to the eyes of normal adult male rats. After persistence for 20 days, only fibrotic nodules were recovered. Previous to the twentieth day, areas of normal-staining donor tissue occasionally could be identified, but there was no histological evidence of the ingrowth of blood vessels from the host. In no instance did these heterotransplanted accessory glands display evidence of secretion. The epithelium of the transplanted vesicular glands was low and void of secretory granules. Histological and cytological preparations indicated the loss of secretory function in the epithelial cells of the prostatic transplants.

Summary. These observations indicate that heteroplastic transplants of gonads and accessory genital glands from albino mice do not become functionally incorporated in the anterior chamber of the eyes of albino rats. It is believed that the serological and phagocytic reactions of the host produce more rapid deterioration of the heterotransplant in the anterior chamber than occurs in other transplantation sites previously studied.

9213 P

Effect of Pneumococcus Type III Specific Polysaccharide on Sedimentation of Blood Cells.

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From the Hygienic Laboratory, University of Michigan.

During the course of experiments with the specific polysaccharide prepared from a type III strain of pneumococcus by the method of Avery, Kendall, and Scherp,[1] it was noted that this material greatly increased the sedimentation rate of citrated human blood.

[1] Heidelberger, M., Kendall, F. E., and Scherp, H. W., *J. Exp. Med.*, 1936, **64**, 559.

This initial finding has been confirmed, and additional observations have been made, which we desire to present in a preliminary paper.

The effect of the S III on the sedimentation rate of blood cells can be demonstrated with heparinized or defibrinated blood, as well as with citrated blood. The effect of concentration of the S III on the sedimentation rate of citrated human blood is indicated by the data in Table I. The mixtures of blood and S III were drawn up in tubes of 2 mm. bore to a height of 200 mm., and the sedimentation read as the height of the clear plasma.

TABLE I.

% concentration of S III	0	.025	.05	.075	.1	.3	1.0%
mm. sedimentation in 60 min.	3	7	21	39	74	174	174 mm.

A carbohydrate substance isolated from a mucoid variant of *B. coli* by C. Lawrence of this laboratory had a similar effect in increasing the sedimentation rate of blood cells. Gelatin acted in the same way, as did gastric mucin, but to a degree much less pronounced than did the specific polysaccharide of the pneumococcus. Glucose in concentrations up to 1% had no appreciable effect on the phenomenon in question.

When mixtures of the S III of the pneumococcus and homologous antiserum are allowed to stand for 15 minutes, and are then added to blood, the increase in sedimentation rate is markedly diminished, as is shown in Table II.

TABLE II.

	Type III Antiserum	Normal Serum	Control no S III
mm. sedimentation in 60 min.	36	74	4

This effect is type-specific, antiserums of heterologous types having no effect.

When 1 mg. of the specific polysaccharide was mixed with 1 cc. of blood, the color of the mixture darkened sooner than the control. On shaking the darkened blood, the color was restored to a bright red, only to darken again before the control. The effect of the carbohydrate on the oxygen-carrying capacity of the blood is being investigated.

Pneumonia is a disease characterized by an abnormally high sedimentation rate. It is also known[2] that the specific polysaccharide may circulate in the blood stream during the course of the disease.

[2] Dochez, A. R., and Avery, O. T., *J. Exp. Med.*, 1917, **26**, 477.

Therefore it seems that the observation here reported may aid in furnishing at least part of the mechanism for the high sedimentation rate seen in pneumonia.

9214

Effect of Phage on Electrokinetic Potential of Susceptible Cells.*

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Earlier work[1, 2] has served to establish the following essential facts concerning the phage-bacterium reaction:

A. Phage-production is conditioned by bacterial growth.

B. There is at all times a normal distribution of phage between susceptible cells and the surrounding medium providing the cells are alive, *i. e.*,

$$\frac{\text{intracellular phage per cell}}{\text{free phage}} = K$$

C. Lysis of bacteria depends upon the attainment of a critical ratio of phage to bacteria. In our experiments this threshold is approximately 100 activity units[3] per bacterium.

While the above relationships are significant for the development of a rational mechanism of phage action on bacteria[4] and have been proved to apply to more than one organism and the corresponding phage,[5] they give no evidence as to how phage induces cellular dissolution. Apparently phage does not measurably alter the normal bacterial growth-rate or the rate of cellular metabolism;[4, 6, 7] it may or may not bring about swelling of susceptible bacteria just before lysis begins. Bronfenbrenner[8] has found that phage produces hydrolytic cleavage of bacterial proteins although

* Supported by grants-in-aid from the National Research Council and American Medical Association.

1 Krueger, A. P., and Northrop, J. H., *J. Gen. Physiol.*, 1930, **14**, 223.

2 Krueger, A. P., *J. Gen. Physiol.*, 1931, **14**, 493.

3 Krueger, A. P., *J. Gen. Physiol.*, 1930, **13**, 557.

4 Krueger, A. P., *Physiol. Reviews*, 1936, **16**, 129.

5 Clifton, C. E., and Morrow, G., *J. Bact.*, 1936, **31**, 441.

6 Eaton, M. D., *J. Bact.*, 1931, **21**, 143.

7 Hallauer, C., *Centralbl. f. Bakt., I. Orig.*, 1933, **130**, 194.

8 Bronfenbrenner, J., Muckenfuss, R. S., and Hetler, D. M., *Am. J. Path.*, 1927, **3**, 562.

this does not seem to be a constant concomitant of phagic action.[9] Since the site of action has never been clearly demonstrated and it is not even known whether phage taken up by a bacterium is inside the cell or merely held on the cell's surface, we felt that measurements of the electrokinetic potential of susceptible staphylococci which had been exposed to anti-staphylococcal phage would be of interest.

I. *Measurement of the rate of electrophoresis.* To estimate the electrokinetic potential the rate of electrophoresis of untreated and phage-treated staphylococci was determined. Two types of suspensions were employed:

A. Live Cells. A suspension of living staphylococci was made from a 16-hour culture of organisms which had been grown on agar, thoroughly washed, and resuspended in sterile water. From this dense suspension the following mixtures were prepared: 12×10^7 staphylococci per ml. in undiluted phage (1×10^{10} activity units/ml.) and 12×10^7 staphylococci per ml. in broth. Both mixtures were kept in ice-water for 2 hours to allow equilibrium between intracellular and extracellular phage to become established. They were then centrifuged, the supernatants decanted, and the discarded solution replaced with an equal volume of secondary sodium phosphate and primary potassium phosphate buffer-mixture of pH 7.0.

B. Dead Cells. A 16-hour culture of staphylococci grown on agar was washed twice with sterile distilled water. The washed cells were made into a dense suspension in sterile distilled water and were heated at 80°C. for one hour. Aliquots were added to broth and to undiluted phage so that the final concentrations in both preparations were 1×10^8 bacteria per ml. The rest of the treatment was identical with the procedure outlined under A.

The rates of migration in the electric field were measured in the Northop-Kunitz microcataphoretic cell[10] and the figures were substituted in the Helmholtz-Lamb equation for calculating the zeta potential. The average electrokinetic potential for living untreated cells was –31 millivolts, while the corresponding figure for phage-treated cells was –59 millivolts. Dead untreated cells had an average electrokinetic potential of –23 millivolts, and dead phage-treated cells an average zeta potential of –46 millivolts.

II. *Test of colloidal stability.* As a further test of the differences in electrokinetic potential engendered by phage, the stability of untreated and phage-treated staphylococcal suspensions to the

[9] Bayne-Jones, S., and Sandholzer, L. A., *J. Exp. Med.*, 1933, **57**, 279.

[10] Northrop, J. H., *J. Gen. Physiol.*, 1921-22, **4**, 629.

addition of thorium nitrate was tested. The bacterial suspensions used were those described above and were prepared both with and without exposure to phage.

In the case of living cells it was found that agglutination was very hard to induce even with high concentrations of thorium nitrate. There was no clear-cut endpoint which would serve to differentiate the untreated and the phage-treated suspensions. Apparently concentrations of thorium nitrate sufficient to reduce the electrokinetic potential below the critical level for agglutination also alter the cohesive properties of the cells.

In carrying out tests with dead bacteria, the cells were prepared as described under I-B with the exception that the final concentrations of bacteria in broth and in phage were 6×10^8 cells per ml. After exposure to phage and to broth for 3 hours the suspensions were centrifuged, the supernatants decanted, and the discarded fluid replaced with equivalent volumes of distilled water. The packed cells were thoroughly shaken up and 0.5 ml. aliquots of each cell suspension were added to 0.5 ml. portions of thorium nitrate dilutions. The suspensions were shaken in the waterbath at 36°C. and were read for agglutination at 0.1-hour intervals. Table I summarizes the general results observed in a series of such tests.

TABLE I.

Effect of phage on stability of heat-killed staphylococcal suspensions exposed to action of thorium nitrate. [Bacteria] = 3 / 10^8/ml. Agglutination expressed as 1+, 2+, 3+, 4+. Reaction carried out at 36° C. with shaking. Higher dilutions were ineffective.

Time (Hrs.)	Broth-Treated Staphylococci				Phage-Treated Staphylococci	
	Normality of Thorium Nitrate					
	1/10000	1/12000	1/16000	1/20000	1/10000	1/12000
0.1	4+	4+	0	0	0	0
0.2	4+	4+	2+	2+	1+	0
0.3	4+	4+	4+	2+	1+	0
0.4	4+	4+	4+	2+	1+	0
0.5	4+	4+	4+	2+	1+	0
0.75	4+	4+	4+	2+	1+	0
1.0	4+	4+	4+	2+	1+	0

Apparently when dead staphylococci take up phage they become quite resistant to the precipitating action of thorium nitrate. It should be noted that the sorption of phage by living staphylococci differs from that observed with dead cells.[2] When living cells are placed in phage-solutions equilibrium between intracellular and extracellular phage fractions is quickly established and maintained in accordance with a partition-coefficient; this attachment of phage to the cell is readily reversible. With dead cells phage distribution is

expressible as an adsorption-isotherm equation and the fraction attached to the bacteria is irreversibly bound.

The attachment of phage to either living or dead susceptible staphylococci increases the negative electrokinetic potential as measured directly by the rate of cataphoretic migration and indirectly by the increased stability of suspensions to the action of quadrivalent cations. This is not proof that phage action is limited to the cell surface but it does furnish evidence that certain of the bacterium's surface-properties are altered either directly as a result of phage-attachment or indirectly as a reflection of intracellular reactions in which cellular constituents and phage participate.

9215 P

Liver Proteins. II. Liver Albumin.

J. MURRAY LUCK AND DOUGLAS MARTIN.

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In the course of investigations on the chemistry of the liver proteins we have had occasion to determine the liver-albumin content under a variety of conditions. The fundamental problem which we desired to elucidate was whether liver albumin exists as a preformed protein or whether it arises as an artefact during fractionation. It occurred to us that a partial answer would be obtained by studying the ratio, albumin/total salt-soluble protein, at different hydrogen ion concentrations maintained during the preliminary sodium chloride extractions. It is known[1] that the total salt-soluble protein extractable from liver increases greatly with increase of pH over the range pH 4 to 8. If the liver albumin were largely an artefact, arising as a dissociation product from the salt-soluble protein fraction, one might reasonably expect that increases in the latter would be reflected by equi-proportional increases in the former; the ratio, albumin/total salt-soluble protein, would remain constant. Although the present work does not give a decisive answer to the question it yields certain information about the liver-albumin content which is pertinent and significant.

For the purposes of this investigation we used dog liver, rapidly perfused *in situ* to remove blood, then excised, frozen with liquid air, powdered, and preserved at −10°C.

[1] Luck, J. M., and Nimmo, C. C., *Proc. Am. Soc. Biol. Chem.*, in press.

In the first group of experiments the procedure followed was essentially that reported previously,[2] except that 3 successive extractions with sodium chloride were employed instead of 2, and the period of dialysis was shortened to 2 days. Extractions were made at pH 4.3, 4.7, 5.0, 6.4, and 7.0. Between pH 4.7 and 7.0 the albumin content remained virtually constant at about 2.2% (extremes 2.07, 2.33); the total salt-soluble protein content increased progressively from 6.44 to 9.18%; the ratio, albumin/total salt-soluble protein, decreased from 0.36 to 0.23.

In the second group of experiments 3 successive extractions were made with 0.5 *M* $(NH_4)_2SO_4$ instead of sodium chloride. The combined extracts were not dialyzed but were immediately salted out by addition of solid ammonium sulphate to the point of half saturation. Albumin was determined in the filtrate gravimetrically after heat coagulation. The extractions were made at pH 4.0, 5.1, 6.3, 7.3, 7.7, and 8.3 and the salting out at pH 6.4. The corresponding values for liver albumin were 0.86, 2.79, 3.47, 3.39, 2.93, and 3.26% respectively; for the total salt-soluble protein, 0.96, 6.93, 10.1, 10.4, 10.3, and 10.2% respectively; and for the ratio of the two, 0.89, 0.42, 0.35, 0.33, 0.29, and 0.32 respectively.

The experiments in the second group reveal that the values for albumin and total salt-soluble protein increase up to pH 6.3 but further increases in pH do not yield higher values. Below pH 6.3 the proportion of the total salt-soluble protein represented by albumin tends to increase but the number of experiments conducted in these more acid regions is insufficient to permit a final interpretation.

We would like to draw attention, also, to the low level at which albumin is maintained in the liver. The highest value we have ever observed is 3.47%. Due to proteolysis which proceeds during dialysis, the albumin values in the first group of experiments are lower than those where dialysis was omitted. Still lower were the albumin values previously reported,[2] where prolonged dialysis was employed.

In the light of these determinations, and on the assumption that serum albumin has its origin in the liver, the slow regeneration of serum albumin during hypoproteinemia may be associated with the low reserves of liver albumin.

[2] Luck, J. M., *J. Biol. Chem.*, 1936, **115**, 491.

9216 P

Responses of Feathers of Male and Female Pheasants to Theelin.*

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Some years ago it was observed[1, 2] that in several species of birds and mammals a number of secondary sex differences, including color, seemed to be dependent on external or hormonal factors and not at all on the genetic sex of the individual. This discovery led to the enunciation of a theory of "equipotentiality" which assumed that male and female tissues are identical in their reactions, particularly to the sex hormones. Among birds the common fowl yielded much in support of this theory, for even in those breeds which show unusual types of reaction, as the Campines,[3] little evidence has been presented to show a difference between the two sexes. Nevertheless minor differences are suggested by the gonadectomy experiments of Finlay[4] and the skin transplantation studies of Masui.[5] In the Reeves pheasant, which belongs in the same family as the domestic fowl, a marked constitutional difference in the behavior of feather follicles of opposite sexes has been found to exist.[6]

An earlier report on these pheasants was concerned only with responses of male and female skin to normal concentrations of sex hormones, which could be tested by the method of skin transplantation. In a further study of Reeves pheasants and also members of the related genus *Phasianus*, it was found that the action of theelin injected intramuscularly is seemingly indistinguishable from that of the normal hormone of the bird's own ovary. Assuming that theelin and the ovarian secretions are essentially identical, the relative responsitivity of male and female feather follicles to the hormone may be compared fairly satisfactorily by means of skin transplantation, gonadectomy and theelin injection. While the

* Supported in part by the Rockefeller Fluid Research Fund of Stanford University School of Medicine. The writer is also indebted to Dr. Oliver Kamm and the Parke-Davis Company for generous contributions of theelin.

1 Zawadowsky, M. M., *Trans. Lab. Exp. Biol.*, Zoopark, Moscow, 1928, **4**, 11.

2 Lillie, F. R., *J. Exp. Zool.*, 1927, **48**, 175.

3 Danforth, C. H., *Biologia Generalis*, 1930, **6**, 99.

4 Finlay, G. F., *Brit. J. Exp. Biol.*, 1925, **2**, 439.

5 Masui, K., *Arch. Entwicklungsmech.*, 1933, **128**, 1.

6 Danforth, C. H., Proc. Soc. Exp. Biol. and Med., 1935, **33**, 291.

results are rather clear cut, there is little doubt that they are obscured somewhat by the coincident action of other hormones than theelin.[7]

The feathers of Reeves pheasants and those of members of the genus *Phasianus* of course differ greatly in actual appearance, but they show sufficient parallelism in their responses to theelin to justify grouping them together for present purposes. In neither group are the reactions of an all-or-none type. Moreover, the responses in different regions of the body vary considerably. Feathers of the head and throat appear to undergo complete "sex reversal" more readily than those of most other regions. In general there seems to be an irregular gradient extending antero-posteriorly, but this and the possible interrelation of male and female hormones require further analysis.

TABLE I.
Approximate Effects of Various Theelin Levels on Developing Feathers of the Rump.

Group No.	Hormonal Condition	Female Follicles		Male Follicles	
		No. of Examples	Response	No. of Examples	Response
1	Castrated ♂	1	3+	4	1
2	Normal ♂	9	3	7	1
3	Castrated ♂ plus theelin	1	4	2	2±
4	Normal ♂ plus theelin	2	3+	6	2± [3+]
5	Castrated ♀	2	1+		
6	Normal ♀	10	4	7	2
7	Normal ♀ plus theelin	3	4	2	2

(+) indicates a variation upward, (±) a range above and below the value indicated.

Table I is based on reactions of feathers of a single region, the rump, where the threshold is rather high and the range of expression wide. As a scale for measuring responses, 4 primary grades may be recognized and designated as 1, 2, 3 and 4, corresponding to M/M, M/F, F/M, and F/F, where numerators represent genotypes and denominators full normal hormonal concentrations. In groups 3, 4 and 7 of the table, injections of theelin in oil averaged a little over 4500 international units a day and were continued for periods of either 4 days or approximately 2 weeks. The former dosage, affected only a part of each growing feather while the latter, when properly timed, was sufficient to influence feathers throughout their entire extent. In group 4 the figure in brackets refers to a specimen of *Phasianus* which was especially sensitive.

It will be apparent that at all concentrations of theelin, from far

7 Witschi, E., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 484.

below to well above normal, female skin consistently produces feathers of a higher grade than male skin at the same concentrations. Even at the extremes reached in these experiments, only an approximation to sex reversal (1+ and 3+) was attained. This indicates that in these species of pheasants the genetic factor is an important one in the determination of sex differences in plumage, and the results afford no support for the theory of equipotentiality as originally formulated.

9217 P

Chilling as an Effective Means of Delousing.

HUEI-LAN CHUNG.

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Louse-borne relapsing fever and typhus fever are prevalent in North China where they often assume epidemic proportions. Since these infections are transmitted by body-lice, delousing will always remain one of the most effective means of controlling them. In China's northern provinces the natural temperature during the months from November to February is stated to fluctuate between –16°C. and –40°C. The problem is to devise an effective, practical, and cheap means of delousing the clothing and other belongings of large numbers of people, especially troops, who are exposed to relapsing fever and typhus fever and live under conditions in which on account of economic and other difficulties, the usual type of delousing by means of moist or dry heat is not available. Hence, a series of experiments has been conducted to test the resistance of body-lice and their eggs to chilling, a natural means which is always available without cost during winter in cold regions.

Altogether 2,922 male and female body-lice varying from 4 days to 32 days old divided into 40 lots, and 2,370 eggs in 9 lots were subjected to chilling at temperatures ranging from –1°C. to –25°C. for various periods of time. Many thousands of lice and their eggs were also subjected to temperatures varying from 5°C. to 8°C. for many days. In most instances the chilling was accomplished by placing the different lots of lice and eggs contained in different cages in a special refrigerator which is adjustable to give any desired temperature from 0°C. to –25°C. In a few instances the lice subjected to chilling were kept in a small test-tube which was closely

wrapped with a thick layer of cotton-wool in all directions. The thickness of this cotton-wool coat measured 3 cm. when uncompressed but measured only 0.5 cm. when compressed. In some instances the chilling was done by merely exposing the lice contained in cages to the outdoor winter temperature and the minimum and maximum temperatures to which the insects were subjected for a given time were recorded with a suitable thermometer.

Death of lice was confirmed by the fact that recovery never took place when these chilled insects were brought back to temperatures between 25°C. and 37°C. In many instances the chilled lice still showed slow and sluggish rhythmic contractions of their stomachs, but they were neither able to move nor ever recovered, and hence can be safely regarded as dead. The results of these experiments may be summarized as follows:

1. Chilling out-of-doors, at a temperature fluctuating between −10°C. and −14°C. for 9 hours, or between −10°C. and −12°C. for 13 hours is lethal to body-lice. This is not in agreement with the observations of Kisskalt and Friedmann[1] and Zabel as cited by Halberkann[2] who stated that chilling at −12°C. overnight or at −15°C. for several days was not lethal to body-lice.

2. Chilling in a special refrigerator at a temperature of −17°C. for 2 hours, or at a temperature of −25°C. for one hour is lethal to body-lice.

3. It requires chilling for 3 hours at a temperature of −17°C. or for 2½ hours at a temperature of −25°C. to kill body-lice when they are protected by a compressed layer of cotton wool 0.5 cm. thick.

4. When fed once daily many body-lice survive a temperature fluctuating from 5°C. to 8°C. for 6 to 8 weeks.

5. Chilling at temperatures between −6°C. and −8°C. for 36 hours or between −10°C. and −11°C. for 10 hours does not kill body-lice. This is in keeping with the observations of Heymann[3] and Hase[4] who found that chilling overnight at −8°C. or −10°C. was not lethal to body lice.

6. Chilling at 5°C. for 7 days or more prevents most of the eggs of body-lice from hatching, but chilling at −17° or at −25°C. for 2 hours effectively prevents all eggs of lice from hatching. This is

1 Kisskalt, K., and Friedmann, A., *Deut. med. Woch.*, 1915, **41**, 397.

2 Halberkann, J., *Arch. f. Schiffs- u. Tropenhyg.*, 1916, **20**, 5.

3 Heymann, B., *Z. f. Hyg.*, 1915, **80**, 298.

4 Hase, A., *Die Biologie der Kleiderlaus.* Original article not available; cited by Da Rocha-Lima and Sikora in ref. 5.

in harmony with the observations of Sikora[5] but does not agree with the findings of Widmann[6] and Kisskalt,[7] who stated that chilling overnight at −5°C. or at −10°C. did not prevent eggs of lice from hatching.

Conclusion. Chilling for a sufficient period is an effective means of destroying both body-lice and their eggs. Where refrigerators having a temperature lower than −12°C. are available, the delousing of valuable furs and other delicate garments which would be damaged by moist or dry heat, can be safely and effectively achieved by this means.

9218

Photodynamic Action of Various Dyes on Bacteria.

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In the presence of ordinary visible light from an electric bulb, methylene blue was found to exert a rapid bactericidal action on certain bacteria which survived the same dye even in higher concentration in the absence of lamplight.[1] At the same time, gram negative bacilli were found to be highly resistant to this action of methylene blue. In continuation of a systematic study of photodynamic action of dyes on bacteria, various other common dyes have been chosen, and tests with representative gram positive and gram negative organisms repeated. The result of such a study is hereby presented.

Saline solutions of eosin, mercurochrome, acid fuchsin, basic fuchsin, and fluorescein, and a commercial 2% solution of trypaflavine were used. Bacteria were grown on either blood- or plain meat-infusion agar for 24 hours and were then suspended in saline. Except in the case of trypaflavine, which was diluted with the suspension to the desired concentrations, suspensions were added to equal parts of dyes in the different dilutions recorded in Table I.

In order to facilitate the study of a large number of specimens at the same time, the procedure previously employed was slightly

5 Da Rocha-Lima, H., und Sikora, H. *Handbuch der Biologischen Arbeitsmethoden*, 1925, **12**, 769.

6 Widmann, E., *Z. f. Hyg. u. Infek.*, 1915, **80**, 289.

7 Kisskalt, K., *Deut. med. Woch.*, 1915, **41**, 154.

1 T'ung, T., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 328.

modified. Instead of petri dishes, sterile, hollow-ground slides with 2 cells each were used. The cells were protected with coverslips which permitted maximal penetration of light from a 100-watt bulb 10 cm. distant. As in previous experiments, the slides were placed on a cooling machine which kept their temperature at or below 20°C. Controls were exposed to diffuse daylight at room-temperature. At intervals of 15, 30, and 60 minutes after exposure, samples were plated and examined for growth after 24 hours' incubation. The pertinent results of the photodynamic action of eosin, mercurochrome and trypaflavine after 60 minutes' exposure to lamplight are presented in Table I, while those from acid fuchsin, basic fuchsin, and fluorescein were omitted as these dyes were practically inert. The low solubility of the last two could account for their inactivity.

Besides the data presented in Table I, it may be mentioned that while 60 minutes of exposure revealed the maximal photodynamic action of the dyes, in the majority of instances however, a 30-minute exposure was almost as effective. Even with 15 minutes' exposure, *Pneumococcus* type I was killed by eosin and by mercurochrome at 1:10,000 dilutions of the dyes, and *C. diphtheriæ* by eosin at 1:10,000. However, the "prozone," in which higher concentration failed to kill while more dilute solutions did, was more noticeable after only 15 minutes of exposure. For instance, while no growth appeared in a suspension of *Pneumococcus* type I in 1:10,000 of eosin, some growth occurred from that of 1:1,000 and maximal growth from those from 1:10 and 1:100. This phenomenon was repeatedly seen in different types of cultures. It seems to suggest that in studying the photodynamic action of dyes on bacteria there is also an optimal dilution at which the dyes would act as it has been previously demonstrated with bacteriophage[2] and with toxin.[3]

Two facts stand out quite clearly from the above observations. In the first place, eosin was found to have a particularly effective photodynamic action—the difference between its native bactericidal action and that after exposure to light was more than 10,000 fold. In the case of methylene blue this difference was only 100 fold. Mercurochrome was more bactericidal in the absence of light, but its action on gram positive organisms was definitely enhanced by light. Trypaflavine behaved in a way intermediate between these 2 dyes. In the second place, it was remarkable that none of the dyes tested would act effectively against a gram negative bacillus, as

2 Perdrau, J. R., and Todd, C., *Proc. Roy. Soc. B*, 1933, **112**, 277.

3 Lin, F. C., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 656.

TABLE I.
Photodynamic Action of Eosin, Mercurochrome and Trypaflavine on Bacteria.

Organisms	Dilution of dye	Eosin		Mercurochrome		Trypaflavine	
		Exp.	Unexp.	Exp.	Unexp.	Exp.	Unexp.
Pneumococcus type I	1:10	+	++++	—	++++		
	1:100	++	++++	—	++++		
	1:1000	—	++++	—	++++	—	++++
	1:10,000	—	++++	—	++++	—	++++
	1:100,000	—	++++	—	++++	++	++++
	1:1,000,000	++++	++++	++++	++++	++++	++++
Streptococcus hemolyticus	1:10	—	—	—	—		
	1:100	—	+	—	+		
	1:1000	—	++	—	++++	+	±
	1:10,000	—	++++	—	++++	+++	++++
	1:100,000	++++	++++	++++	++++	++++	++++
	1:1,000,000	++++	++++	++++	++++	++++	++++
Staphylococcus albus	1:10	++++	++++	—	—		
	1:100	++++	++++	—	—		
	1:1000	++++	++++	—	—	—	++
	1:10,000	++++	++++	—	++++	—	++++
	1:100,000	++++	++++	++++	++++	+	++++
	1:1,000,000	++++	++++	++++	++++	++++	++++
S. paratyphi	1:10	++++	++++	—	—		
	1:100	++++	++++	—	—		
	1:1000	++++	++++	—	+	—	+
	1:10,000	++++	++++	+++	++++	+++	+++
	1:100,000					++++	++++
	1:1,000,000					++++	++++

C. diphtheriæ	1:10	—	++++	—	—		
	1:100	—	++++	—	—		
	1:1000	—	++++	—	—	—	+
	1:10,000	—	++++	—	++++	—	+++
	1:100,000	—	++++	++	++++	±	+++
	1:1,000,000	++++	++++	++++	++++	+++	+++
S. paradysenteriæ Flexner	1:10	++++	++++	—	—		
	1:100	++++	++++	—	—		
	1:1000	++++	++++	—	—	—	—
	1:10,000	++++	++++	++++	++++	—	++++
	1:100,000	++++	++++			++	++++
	1:1,000,000					++++	++++
Neisseria intracellularis	1:10	—	—	—	—		
	1:100	—	++++	—	—		
	1:1000	—	++++	—	++	—	—
	1:10,000	—	++++	—	++++	—	—
	1:100,000	—	++++	++++	++++	—	+++
	1:1,000,000	++++	++++	++++	++++	+++	+++
Br. abortus	1:10	++++	++++	—	—		
	1:100	++++	++++	—	—		
	1:1000	++++	++++	—	—	—	—
	1:10,000	++++	++++	++++	++++	—	—
	1:100,000			++++	++++	—	++
	1:1,000,000			++++	++++	++++	++++

— = no growth.
\+ = growth of less than 5 colonies.
++ to ++++ = increasing number of colonies.

the maximal difference between the native bactericidal action of dyes and that enhanced by light was at most 10 fold. It therefore appears that the parallelism of bacteria in their reactions to the gram stain and the susceptibility to photodynamic action, as suggested previously,[1] is again confirmed.

9219

Attempts to Infect *Ornithodorus moubata* with the Chinese Strain of *Spirochaeta recurrentis.*

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Biologically there are 2 groups of relapsing fever spirochaetes, one being transmitted by lice and the other by ticks, chiefly of the *Ornithodorus* group. The Chinese strain of *Spirochaeta recurrentis* has been demonstrated by Robertson[1] and Chung[2] to be transmitted by the louse, *Pediculus humanus corporis,* and its development in this insect has been studied recently by Chung and Feng.[3] In order to see whether infection with the Chinese strain of relapsing fever spirochaetes can be established in *Ornithodorus moubata,* and whether infection can be transmitted to laboratory animals by these ticks, a series of experiments was carried out.

Four lots of young larval ticks and one lot of adult male and female ticks were fed on squirrels heavily infected with Chinese relapsing fever (30-40 spirochaetes to each oil immersion dark field of the fresh smear). After the infective feeding, the ticks were kept at a room temperature of 25-28°C. Examinations for infection of the ticks were made by dissection and searching for spirochaetes under darkfield illumination by the method used by Feng and Chung,[4] by feeding on clean squirrels and finally by injecting emulsified ticks into squirrels. The blood of the animals was examined daily with dark ground illumination for spirochaetes throughout the course of the experiment.

The dissections, as shown in Table I, demonstrate that the spiro-

1 Robertson, R. C., *Chinese Med. J.*, 1932, **46**, 853.
2 Chung, H. L., *Chinese Med. J.*, 1936, **50**, 1723.
3 Chung, H. L., and Feng, L. C., *Chinese Med. J.*, 1936, **50**, 1181.
4 Feng, L. C., and Chung, H. L., *Chinese Med. J.*, 1936, **50**, 1185.

TABLE I.

Results of Dissection of Ticks Fed on Squirrel Infected with Chinese Strain of *Spirochæta recurrentis*. (These ticks were kept at 25-28° C.)

Days of Infection	No. Dissected	Stomach		Legs		No. of spirochaetes	
		No. +	No. —	No. +	No. —	Living	Dead
1	6	6	0	3	3	++++	+
2	8	8	0	1	7	++	+++
3	8	8	0	1	7	+	++
4	4	3	1	1	3	+	++
5	5	2	3	0	5	0	+
6	4	0	4	0	4	0	0
7	5	0	5	0	5	0	0
8	2	0	2	0	2	0	0
9	2	0	2	0	2	0	0
10	2	0	2	0	2	0	0

chaetes penetrated the stomach wall and reached the body cavity in some of the ticks during the first 4 days of infection. All the spirochaetes, including those which had already reached the body cavity and those still in the stomach gradually died, degenerated, and finally disappeared, beginning as early as the 4th day in some of the ticks. In a few instances, a small number of spirochaetes were found to survive until the fourth day of infection. From the 6th day onward spirochaetes could not be found at all in any of the ticks examined. In a few of the ticks dissected from the 1st to the 11th day of infection additional examinations for spirochaetes in other organs lying in the body cavity, including the salivary glands, the nerve ganglion, the coxal glands, the reservoir, and the malphighan tubes gave entirely negative results.

In the feeding experiments, since the ticks refused to suck blood again earlier than 4 days after the infective feed, and even after this date only a small number of them would suck blood at the same time, these experiments were extended over a period of 25 to 69 days in the hope that repeated bites during this period might produce an infection which would otherwise be missed.

In Table II it is shown that all 4 squirrels on each of which separate lots of 5-17 infected ticks were fed from 3-6 times with a total of 45-66 bites failed to contract relapsing fever and their blood remained constantly negative for spirochaetes as proved by daily examination extending 31-55 days after the last feed.

At the end of the feeding experiment 5 ticks from each of the 4 lots were ground up in 2 cc. normal saline solution and the emulsion injected subcutaneously into the negative squirrels used for the feeding experiment. Besides the subcutaneous injection, 4 drops of the emulsion were also dropped into the eyes and nose of each animal. On examination, 8 and 25 days respectively after the injection, 2

TABLE II.
Result of Feeding and Inoculation Experiments.

	Feeding of ticks on squirrels					Injection of emulsified ticks into squirrels		
Squirrel No.*	Days after ticks fed been infected	No. of feedings	No. of ticks fed each time	Total No. of bites	Blood of squirrel	No. of ticks injected	Animals remained negative after injection	
1	66-116	5	9-12	52	No spirochaetes	5	25 days.	Died
2	7-32	4	8-14	45	" "	5	35 "†	
3	19-58	3	14-17	45	" "	5	34 "†	
4	4-73	6	5-15	66	" "	5	8 "	Died

*All the squirrels were splenectomized.

†On the last day 4 drops of infective blood were put into the eyes and nose of each of these squirrels, in whose blood spirochaetes were found 4 and 5 days afterwards.

animals which died showed no spirochaetes in the blood. Into the eyes and nose of the other 2 squirrels, whose blood was consistently negative for 33 and 34 days after receiving the tick emulsion, infected squirrel blood was dropped. They were found to take the infection, as spirochaetes were seen in their blood 4 or 5 days afterwards. This shows clearly that the squirrels used in the experiment were susceptible to the infection (Table II).

Attempts to transmit relapsing fever spirochaetes which are normally carried by lice by means of ticks to laboratory animals have been made by various workers. Manteufel[5] stated that he was able to transmit the Russian strain of relapsing fever spirochaetes to rats through the bite of experimentally infected *Ornithodorus moubata*. Neumann[6] also succeeded in transmitting both the American and Russian strains of *Spirochaeta recurrentis* to mice and rats through the bite of the same tick.

In the experiments recorded in the present paper infection with the Chinese strain of *Spirochaeta recurrentis* of Peiping could not be maintained in either young or adult *Ornithodorus moubata*. After the infective feed, some of the spirochaetes transversed the gut and reached the abdominal cavity of the tick. They failed, however, to enter the salivary glands, the nerve ganglion, the reservoir and the coxal glands.

At the temperature of 25-28°C. the spirochaetes in the stomach as well as those that had already reached the body cavity of the tick gradually died, degenerated and finally disappeared about 6 days after the infective feed. Feeding of such ticks on and injection of their emulsion into squirrels failed to produce relapsing fever in these animals which were subsequently proved to be susceptible.

These findings suggest that the Chinese strain of *Spirochaeta recurrentis* is biologically different from those transmitted by ticks. It also seems to indicate that this strain of the relapsing fever spirochaetes does not behave in the tick in the same way as the Russian and American strains which, according to the work of Manteufel and Neumann, can be easily transmitted to laboratory animals by *O. moubata*.

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5 Manteufel, Arb. a. d. Kaiserl., *Gesundheitsamte*, 1908, **29**, 337.

6 Neumann, R. O., *Münch. Med. Wochr.*, 1909, **56**, 477.

9220 P

Negative Findings of Leucolysin in the Sera of Cases of Kala-azar.

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Leucopenia is one of the outstanding features in cases of kala-azar. Its pathogenesis is still obscure. By incubating washed leucocytes of the blood of normal individuals with the sera of other normal individuals and of cases of kala-azar, Maggiore and Sindoni[1] found that there was a reduction in number of leucocytes with kala-azar serum, but no reduction with the normal serum. They, therefore, concluded that in the serum of kala-azar cases there was a leucolytic substance, "leucolysin," which is responsible for the occurrence of leucopenia. Their observation was later confirmed by other Italians,[2,3] but Teng and Forkner[4] showed that inoculation of killed *Leishmania donovani* into rabbits failed to produce leucopenia in these animals. In order to confirm or disprove the conclusion of the Italian workers we repeated their experiments and the results are briefly reported below.

Two cc. of blood was withdrawn from the arm vein of a normal individual. After a drop of blood from the tip of the needle was taken, immediately following its withdrawal from the vein, for the initial count of the white blood cells, the blood in the syringe was quickly forced into a 15 cc. centrifuge tube containing 12 cc. of citrated normal saline. The mixture was thoroughly stirred by a glass rod and centrifuged for 3 minutes at medium speed. The supernatant fluid was then removed, fresh citrated saline was added, the mixture again thoroughly stirred, and at the end of the third centrifugation the sediment was made up to its original volume of 2 cc. with citrated normal saline. One cc. of this blood cell suspension in saline was now put into each of 2 small tubes and another white blood cell count was made. After this 2 drops of kala-azar serum (obtained from etiologically proven but untreated case of kala-azar) were added to one tube and 2 drops of normal serum (obtained from another normal individual whose blood belongs to the same group as that of the kala-azar patient and the individual

1 Maggiore, S., and Sindoni, M., *La Pediatria*, 1917, **25**, 81.

2 Jemma, G., *La Pediatria*, 1920, **28**, 1081.

3 Arena, G., *La Pediatria*, 1927, **35**, 465.

4 Teng, C. T., and Forkner, C. E., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 661.

supplying the 2 cc. of blood) were added to the other. Another white count was then made. The tubes, now properly corked, were then incubated in a water bath for 2 or 3 hours and white counts were made at hourly intervals from each tube. The blood was thoroughly stirred with a glass rod before the sample was taken for each counting.

The above experiment was made with the serum of 6 cases of kala-azar. It was observed that in all these cases there was always a reduction in the number of leucocytes after several times of washing and after prolonged incubation with repeated stirring. The degree of reduction in the number of the leucocytes of the blood incubated with kala-azar serum was approximately the same as that with normal serum. The reduction was apparently due to the fact that many of the leucocytes in the suspension became attached to the stirring rods and the wall of the incubating tubes, as demonstrated by the microscopic examination of the stained blood films on their surfaces and further proved by the fact that with very thorough cleansing or paraffin coating of the glassware the degree of reduction became much less noticeable. It was, therefore, concluded that in the kala-azar serum there is no such substance as leucolysin. The lack of any leucolytic substance was further confirmed by the direct observation under microscope of the leucocytes of normal blood mixed with kala-azar serum. These leucocytes were found to remain either actively motile or at least physically intact even after 3 hours' incubation.

9221 P

Pigmented Cells in Adrenals and Testes of Hypophysectomized Rats.

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From the Department of Biochemical Research, Cleveland Clinic Foundation, Cleveland, Ohio.

Brief mention has already been made of the fact that hypophysectomy in the rat is followed by the appearance of yellowish pigmented cells in the interstitial spaces of the testis and in the reticular zone of the adrenal.[1] The pigmented cells which we have seen in the testis seem to be similar to those described by Rasmussen[2] as occurring in

1 Cutuly, E., McCullagh, D. R., and Cutuly, E. C., to appear in *Am. J. Physiol.*

2 Rasmussen, A. T., *Am. J. Anat.*, 1917, **22**, 475.

the involuted testis of the hibernating woodchuck, and those we have seen in the adrenal seem to be similar to those found by Zalesky[3] in the adrenals of castrated guinea pigs.

In our studies the adrenals and testes of numerous rats hypophysectomized for 5 to 100 days were fixed in Zenker-acetic, 10% formalin, Helly's or Bouin's fluid. Paraffin sections were cut at 5 or 10 micra and stained with ordinary hematoxylin-eosin or with iron hematoxylin and light green; some sections were mounted unstained. The structure and coloration of the pigmented bodies was the same regardless of the histological technique employed. These bodies were of different sizes and shapes and were characterized by the appearance of yellow pigment in the cytoplasm. The size of the pigment granules varied from fine particles to clusters as large or larger than nuclei of normal interstitial cells. Nuclei were sometimes entirely lacking from these cells. When present they showed varying degrees of pycnosis and were almost always eccentrically located, their position seeming to depend upon the amount of pigment laid down in the cytoplasm. At times this pigment was so abundant that the nucleus was displaced to the extreme periphery of the cell and actually protruded beyond the cell wall.

Pigment cells such as we have described are very rarely observed in the adrenals of normal rats, but in our experience never appear in the testes. Such cells have been found to occur invariably in the juxtamedullary region of the adrenals of rats hypophysectomized from 5 to 100 days. Greater numbers of these cells were visible in the adrenals of rats hypophysectomized for relatively long periods of time than in those hypophysectomized for only a short time. This observation suggests that there was accumulation of these structures in the reticular zone. In the testes the pigmented cells appeared indiscriminately throughout the intertubular spaces, but they were usually most numerous just subjacent to the tunic of the gland. While they have been seen in the testes of rats hypophysectomized for 5 to 69 days, their occurrence in these organs was not so constant as in the adrenals. Frequently these yellow bodies were few in number or entirely lacking in the testes of rats hypophysectomized for more than 20 days. Since these pigmented cells in hypophysectomized rats usually showed signs of nuclear atrophy, and because they accumulated in the reticular zone of the adrenal and tended to disappear from the interstitial spaces of the testis, it was believed that they represented a type of degeneration resulting after ablation of the anterior lobe of the pituitary. That removal of the anterior

[3] Zalesky, M., *Anat. Rec.*, 1936, **65**, 467.

pituitary alone was responsible for the appearance of the pigmented cells was demonstrated by the fact that the testes and adrenals of incompletely hypophysectomized rats possessing remnants of anterior lobe tissue alone did not show these changes.

Because of the morphological similarity between the pigmented structures in the adrenals and testes, it was thought that the cells in both the glands might be physiologically related. Various studies, however, have not lent support to this idea. Thus, hypophysectomized rats receiving pure gonadotropic hormone from a parabiotic partner[4] had testes which showed no sign of pigmented cells, while the adrenals of these rats contained many such structures. Similarly, pigmented cells have been prevented from appearing in, or have been caused to disappear from, the testes of hypophysectomized rats by injections of antuitrin-S; but these injections failed to have any preventive action on the pigmented bodies in the adrenal.

Further studies are being planned to determine the nature, origin and fate of the pigmented cells and their responses to various substances.

Summary. Hypophysectomy in the rat caused invariably in the reticular zone of the adrenal and sometimes in the intertubular spaces of the testis the formation of cells containing yellow pigment granules. The nuclei of the cells usually showed varying degrees of pycnosis or were absent. Gonadotropic hormone prevented or corrected these changes in the testis, but failed to have any apparent effect upon the pigmented structures in the adrenal.

9222

A Comparison of the Potencies of Some Androgenic Sterols.

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This is a report of a study of the relative effects of six synthetic androgens* on the combs of capons. Some of these androgens had not been assayed previously by the procedure of Gallagher and Koch[1] which, in our hands, has proved to be the most satisfactory

[4] Cutuly, E., McCullagh, D. R., and Cutuly, E. C., *Endocrinology*, 1937, **21**, 241.

* These substances were supplied through the courtesy of Dr. E. Schwenk of the Schering Corporation.

[1] Gallagher, T. F., and Koch, F. C., *J. Pharm. and Exp. Therap.*, 1935, **55**, 97.

method from the point of view of both convenience and accuracy. It must be remembered that the relative potency of these androgens would probably not appear to be the same if other biological test objects were employed. Since, however, the international androgenic unit has been defined in terms of growth of the capon's comb, it is for the present necessary to compare various products by this method.

Since it is known that a single injection of any of these sterols produces results which last for a rather short period, it seemed possible that some of the testosterone derivatives might have a more protracted action and thus prove much more satisfactory for clinical use than unmodified testosterone. Two of the critical points in the testosterone molecule are the 3 and the 17 carbon atoms since the keto and the hydroxy groups are found in these positions. The position of the unsaturated linkage also seems to be of significance in regard to the activity of these derivatives. The compounds employed in this investigation were chosen with these points in view.

The results of assays by the method of Gallagher and Koch[1] are recorded in Table I. According to this method of study, the formation of the oxime definitely decreases the potency of the 2 very active compounds testosterone and testosterone propionate. These oximes apparently are not readily metabolized to ketones by the capon. That the propionate is very active was not surprising since it has been formerly shown by Parkes[2] and others that, in the rat, testosterone propionate and testosterone acetate were many times more effective than the free hormone. This is probably due to the fact that after absorption the ester is slowly metabolized with the formation of the free hormone, thus giving the same effect as the frequent administration of hormone. The androstenediol employed was the Δ^5 androstenediol-3, 17. The fact that a very large dose produced only a very small comb-growth cannot be due to the fact that

TABLE I.
Results of Assay of the Androgens.

Androgen used	Daily dose per bird in micrograms	Average comb-growth (Corrected to 57 mm.)	Calculated micrograms per bird unit
Androsterone	100	8.4	100
Testosterone	15	10.1	11
Testosterone propionate	15	8.0	17
Testosterone oxime	60	6.0	100
Testosterone propionate oxime	30	5.8	53
Androstenediol	250	7.2	310

[2] Parkes, A. S., *Lancet*, 1936, **2**, 674.

the double bond is in a different position than that of testosterone since the Δ^4 androstenediol-3, 17 has been shown to have little if any activity in the capon test.

It seemed possible that further information might be gained by the injection of these compounds in larger single doses. Capons were therefore injected with single doses of 10-bird units of each substance and the curve of growth and regression was measured. In each case the dose was dissolved in one cubic centimeter of sesame oil and injected into the pectoral muscles. Five birds were used in each test and all substances were tested under the same conditions. The results are graphically summarized in Fig. 1.

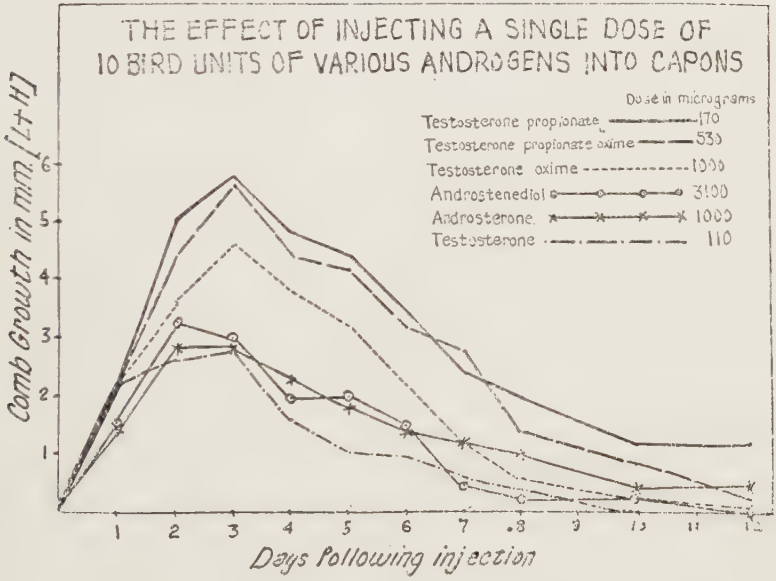

FIG. 1.

Since there was no sign of activity after the third day, it would seem that in every case the material was absorbed and either excreted or inactivated by that time. In the assay of androsterone by the Gallagher and Koch procedure, the injection of one unit on each of 5 consecutive days caused a growth of 8.4 mm., whereas the greatest growth obtained (using an average of 5 birds) with a single dose of 10 units was less than 6 mm. The 2 testosterone esters injected in single doses were much more effective than were the other compounds. The curves for testosterone, androsterone, and androstenediol were either flat or nearly so after the second day, the curves for the oxime, the propionate, and the propionate oxime

of testosterone all continued to show considerable growth between the second and third days. This protracted action would indicate that the esters are the more practical compounds for clinical use.

Summary. Androsterone, testosterone, testosterone propionate, testosterone propionate oxime, testosterone oxime, and androstenediol have been compared in regard to their effect on the combs of capons.

9223 P

Active Sensitization of White Mice.

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According to the only known studies dealing specifically with active anaphylaxis in mice,[1, 2, 3] a certain number of mice given a single prior injection of horse serum may be expected to show anaphylactic reactions when reinjected after the usual incubation period, but a higher proportion of the animals will react if 2 sensitizing injections have been made. In an attempt to attain the maximum degree of sensitivity, we gave different groups of normal mice, one 2, 3, or 4 intraperitoneal or intravenous doses of horse serum or egg white. The mice were then tested for sensitivity by the intravenous or intraperitoneal injection of the appropriate antigen at varying intervals after the last sensitizing dose, but usually after 12-21 days.

The results observed with mice sensitized to horse serum and tested by intravenous doses ranging between 0.5 cc. and 1.5 cc. are summarized in Table I. The effect of repeated sensitizing injections in increasing the liability to severe anaphylactic reactions seems clearly indicated. Only in groups of mice that received 3 or 4 antigen doses prior to the shocking dose were reactions observed in all the animals, and the best results were obtained with mice given 4 sensitizing inoculations. Severe or fatal reactions occurred in only about 40% of mice sensitized by 2 injections, but this percentage increased to 89% in the mice sensitized by 4 injections. In the latter group occurred the highest proportion of deaths (48%).

1 Braun, H., *Munchener med. Wochenschr.*, 1909, **37**, 1880; *Z. f. Immunitats.*, 1910, **4**, 590.

2 Ritz, H., *Z. f. Immunitats.*, 1911, **9**, 321.

3 Von Sarnowski, *Z. f. Immunitats.*, 1913, **17**, 577.

TABLE I.
Effect of Repeated Sensitizing Injections on Anaphylactic Reactions in White Mice.*

Sensitizing injections (horse serum)		Days incubation	Total inoculated	Anaphylactic reactions						No. symptoms	
				Fatal		Severe		Mild			
No.	Amt. and route			No.	(%)	No.	(%)	No.	(%)	No.	(%)
One	0.5cc. i.p.	21	6					1	(17)	5	(83)
	0.5cc. i.v.	23	6	1	(17)	1	(17)			4	(66)
Two	0.3cc. i.p. 0.5cc. i.p. in 5 days	9-21	23	7	(30)	2	(9)	10	(44)	4	(17)
	0.5cc. i.v. 0.5cc. i.v. in 5 days	14	17	2	(11)	5	(30)	9	(53)	1	(6)
	0.5cc. i.p. 1-2cc. i.v. in 21 days	13	13	4	(31)	2	(15)	5	(39)	2	(15)
Three	0.3cc. i.p. 0.5cc. i.p. 0.5cc. i.v. 5 days apart	14-23	18	1	(6)	8	(44)	9	(50)		
	0.5cc. i.v. 0.5cc. i.v. in 4 days 1-2cc. i.v. in 14 days	31-38	17	5	(29)	4	(24)	7	(41)	1	(6)
Four	0.5cc. i.p. 0.5cc. i.p. 0.3cc. i.p. 0.1cc. i.p. 5 days apart	12-16	27	13	(48)	11	(41)	3	(11)		

*All mice tested for sensitivity by intravenous injections of from 0.5cc. to 1.5cc. horse serum

Intravenous and intraperitoneal injections apparently sensitized equally well.

Essentially similar results were obtained with mice sensitized to egg white.

Sensitivity was demonstrated as early as the 9th day, and was shown to persist at least as late as the 40th day, after the last of 2 or more sensitizing injections. Neither the number nor the severity of reactions was apparently influenced by varying the incubation period between the 12th and the 23rd day.

In agreement with the early reports[2, 3] it was found that intraperitoneal injection of the test dose of antigen usually produced no symptoms at all (Table II), although occasionally a typical fatal shock was observed. Intravenous injection of the shock test dose, on the other hand, regularly caused some degree of reaction in mice that were sufficiently sensitized. These inoculations were made into a tail vein, and the typical anaphylactic reactions appeared only

when the injection was well done, so that the entire inoculum was introduced rapidly into the circulation.

From the data presented in Table II it is evident that the degree of sensitization of the animals was a more important factor in determining the severity of the reactions than the size of the intravenous shocking dose. The highest percentage of severe illness occurred among those mice sensitized by 4 intraperitoneal injections and tested by an intravenous dose of from 0.5 cc. to 1.0 cc. Of the 30 mice so treated 15 (50%) died, 12 (40%) were made severely ill, and the remaining 10% showed mild but definite reactions. All of the 18 mice within this group which were given the 1.0 cc. test dose had severe reactions, and 61% of them died in acute shock.

TABLE II.

Effect of Size of Shocking Dose and Route of Injection on Anaphylactic Reactions in White Mice.

Number of previous injections	Shocking dose cc.	route	Total inoculated	Anaphylactic reactions: Fatal No. (%)	Severe No. (%)	Mild No. (%)	No. symptoms No. (%)
1	0.5	i.v.	2				2 (100)
	1.0	"	6	1 (17)	1 (17)		4 (66)
	1.5	"	4			1 (25)	3 (75)
	2.0	"	6				6 (100)
	1-3.5	i.p.	10				10 (100)
2	0.3-0.5	i.v.	11	1 (9)	1 (9)	5 (46)	4 (36)
	1.0	"	16	3 (19)	2 (13)	10 (62)	1 (6)
	1.5	"	26	9 (35)	6 (23)	9 (35)	2 (7)
	2.0	"	2			2 (100)	
	1-3.0	i.p.	30	1 (3)		7 (23)	22 (74)
3	0.3-0.5	i.v.	9		5 (56)	3 (33)	1 (11)
	1.0	"	30	7 (23)	8 (27)	15 (50)	
4	0.05	"	4	1 (25)		3 (75)	
	0.1	"	5			5 (100)	
	0.2	"	5			5 (100)	
	0.3	"	5	2 (40)	1 (20)	2 (40)	
	0.4	"	8	3 (38)	4 (50)	1 (12)	
	0.5	"	8	4 (50)	2 (25)	2 (25)	
	0.7	"	4		3 (75)	1 (25)	
	1.0	"	18	11 (61)	7 (39)		
	1.5	"	2	1 (50)		1 (50)	
	0.5-1.5	i.p.	12			2 (17)	10 (83)

These results apparently represent the best that may be expected with mice, at least with the procedures used. Fatal anaphylactic shock is evidently not surely predictable with any test dose, but the great majority of fully sensitized mice will show a characteristic severe illnes on the intravenous injection of the antigen.

The reactions in the sensitized mice were shown to be specific, and truly anaphylactic in nature, by numerous control experiments.

9224

A Note on the Respiration of *Bacillus coli.**

F. L. Wynd. (Introduced by J. Bronfenbrenner.)

From the Laboratories of the Department of Bacteriology, Washington University School of Medicine.

The respiration of prepared aliquots of a suspension of *Bacillus coli* has been used extensively in this laboratory for comparing the calibration-constants of different Barcroft-Haldane differential manometers and their culture-vessels. The great accuracy of the observations has brought to light an hitherto unreported phenomenon in the oxygen-absorption of this organism.

The suspension of bacteria grown on agar for 18 hours was washed off with 10 cc. of broth, filtered through sterile cotton, and 0.1 cm. of the resulting suspension was placed in the vessel of the respirometer after further diluting it with 10 cc. of broth. The oxygen-uptake was observed at this final dilution at 37°C. Plating showed the cultures to be pure at the end of the experiment.

It was found that the rate of oxygen-uptake increased logarithmically, as would be expected, until the culture reached the age of about 3 hours and 40 minutes. At this time a new rate was initiated. The course and magnitude of this change is seen in Table I and plotted on Fig. 1. Many similar experiments were carried out, all of which showed the break in the curve at very nearly the same age (Table III).

The results tabulated in Table III were obtained with different manometers and in various types of culture-vessels. These experi-

TABLE I.
Manometric Data for Fig. 1.

Time, min.	$-O_2h$*	Time, min.	$-O_2h$*
95	0.0	240	87.9
105	.5	245	93.0
115	1.6	255	104.5
190	34.5	265	116.2
197	42.6	275	128.8
205	52.7	285	141.1
215	67.7	295	152.4
220	71.7	305	156.1
225	74.8	315	158.6

*$-O_2h$ = difference in height in millimeters of the meniscus in the 2 arms of the manometer. Observations begun after 95 min.

* This investigation was supported by the grant of the Rockefeller Foundation to Washington University for Research in Science.

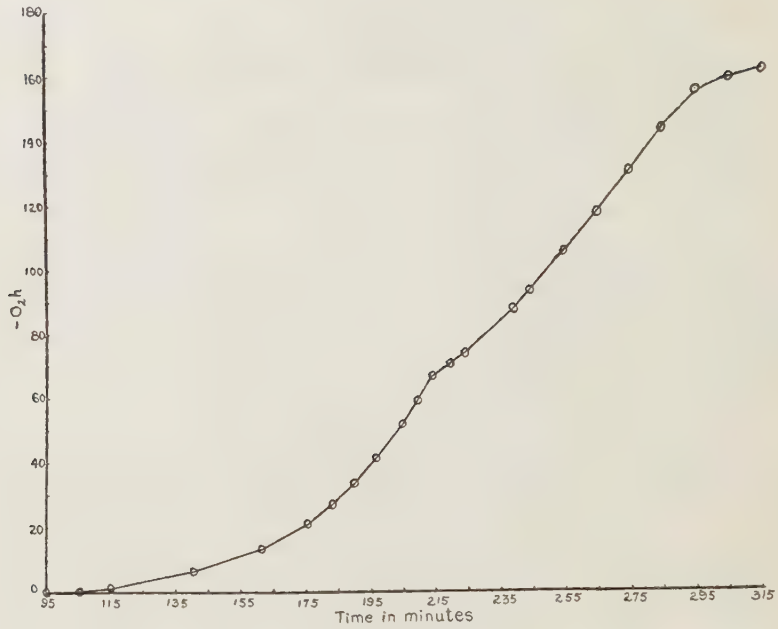

FIG. 1.

This figure shows the time of the break in the rate of oxygen use of *Bacillus coli*. See Table I for data.

TABLE II.
Manometric Data for Fig. 2.

Time, min.	$-O_2h$*	Time, min.	$-O_2h$*
120	0.0	186	56.9
130	4.7	188	60.0
135	5.9	190	62.9
140	6.7	192	65.4
145	9.0	194	66.8
150	13.4	196	68.3
160	24.4	198	69.8
165	28.7	200	71.4
175	40.9	210	80.0
182	50.9	220	89.9
184	53.8		

*$-O_2h$ = difference in height in millimeters of the meniscus in the 2 arms of the manometer. Observations begun after 120 min.

ments were carried out at irregular intervals over a period of several months during which time many different batches of broth were used.

In order to make sure that the break in the curve would be as sudden as manometric readings at 10-minute intervals indicated, the readings were made at intervals of 2 minutes. From data presented in Table II and plotted on Fig. 2, it may be seen that the transition in rates surprisingly occurred within a 2-minute interval.

TABLE III.

The Age of Various Cultures of *Bacillus coli* at which the New Rate of Oxygen-use is Initiated. All Cultures Carried at 37° C.

Hours	Minutes	Hours	Minutes
4	0	3	15
3	48	3	26
3	25	3	40
3	45	3	30
3	30	3	45
3	40	3	45
3	55	4	0
3	45	3	30
3	27	3	40
3	50		

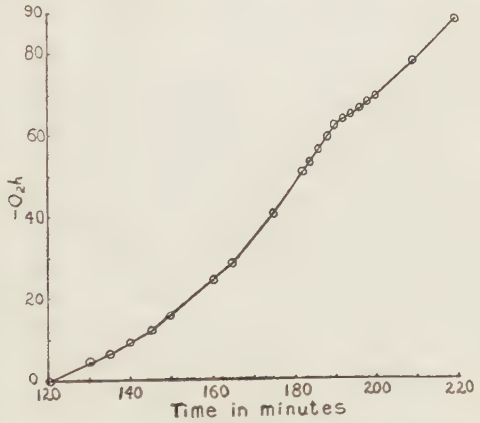

FIG. 2.

The break in the curve occurs within a 2-minute interval. See Table II for data.

It is obvious from the small magnitude of the change in the rate of oxygen-uptake that the highest accuracy in procedure is necessary for its observation. Errors as small as 2 to 3% would effectively mask it. The apparatus used was the Warburg waterbath, vigorously stirred and the temperature controlled to ±.001°C. by a vacuum-tube relay. The manometers were made from selected glass capillaries of a smooth and even bore. The manometer-fluid was triply distilled kerosene stained with Sudan III. Repeated use of the present equipment by several workers in this laboratory has shown that the oxygen-uptake of a culture may be determined with an experimental variation of less than ±0.5%.

The fact that the culture of *Bacillus coli* exhibits these 2 cycles of respiratory activity should be of interest to the students of bacterial respiration. Since conditions do not permit immediate investigation of the nature of this phenomenon by the author, it is here reported in the hope that others will attempt its elucidation.

9225

Influence of Extracts of Anterior Lobe of Pituitary on Glucose Oxidation and Glycogen Storage.

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It was mentioned previously[1] that injection of an anterior lobe extract decreased very markedly the amount of carbohydrate oxidized by normal rats following glucose feeding. The present experiments were undertaken in order to see what was the fate of the glucose which failed to be oxidized.

Male rats* were fasted for 24 hours and were given an intraperitoneal injection of one cc. of anterior lobe extract† 1½ to 3 hours prior to the feeding of a known amount of glucose by stomach tube. The respiratory metabolism was measured for 3 hours after the glucose feeding by the gravimetric procedure of Haldane. The animal was then quickly anesthetized by injection of pentobarbital. The 2 gastrocnemii were removed for separate glycogen analyses, a blood sample was drawn from the *vena cava,* the gastrointestinal tract was removed for the determination of the amount of glucose which had not been absorbed and finally the entire liver was used for glycogen analysis. Details of the analytical procedures used are described in a previous paper.[1] Control animals were given an intraperitoneal injection of one cc. of extract heated previously for 15 minutes in boiling water. In a second series of experiments the same extract injections were given and in addition 0.05 units of insulin per 100 gm. rat were injected subcutaneously immediately before the glucose feeding.

The data in Tables I and II indicate that the animals injected with the active extract oxidized less glucose and deposited more liver and muscle glycogen than did the controls injected with the heat-inactivated extract. The amounts of liver and muscle glycogen and blood sugar present before the glucose feeding were determined in a series

[1] Fisher, R. E., Russell, J. A., and Cori, C. F., *J. Biol. Chem.*, 1936, **115**, 627.

* The rats used were of the Wisconsin strain and were obtained through the courtesy of Dr. Neff of Anheuser-Busch, Inc., after having been used for vitamin B assay.

† The extract was prepared by L. J. Wade from beef anterior lobes by the method of Evans, Cornish and Simpson.[2] The same extract was used for all experiments; it was kept frozen in small individual bottles until used.

[2] Evans, H. M., Cornish, R. E., and Simpson, M. E., Proc. Soc. Exp. Biol. and Med., 1930, **27**, 101.

TABLE I.
Average Values Obtained 3 Hours after Glucose Feeding.

No. of rats	Body wt., gm.	Liver wt., gm.	Blood sugar, mg. %	Liver glycogen, %	Muscle glycogen,* mg. %	O_2 used cc./100 gm./hour	Respiratory quotient	Remarks
9	120	5.89	204	2.99 ±0.55	940 ±145	188	.774	1 cc. active extract
7	130	5.95	125	2.24 ±0.23	798 ±150	175	.879	1 cc. heated extract
6	126	5.75	171	3.48 ±0.38	939 ± 76	196	.775	1 cc. active extract plus .05 units insulin
7	128	5.72	125	2.69 ±0.57	887 ±142	162	.898	1 cc. heated extract plus .05 units insulin

*Average of right and left gastrocnemius.

TABLE II.
Calculated from Table I. All values are given in mg. per 100 gm. rat per 3 hours.

No. of rats	Glucose retained in: Blood and tissue fluids	Liver	Muscle	Glucose oxidized	Total	Glucose absorbed	% accounted for
9	57	142	253	147	599	940	63.7
7	17	98	182	387	684	933	73.3
6	40	154	252	159	605	884	68.4
7	17	116	226	405	764	945	80.8

of control animals‡ fasted previously for 24 hours. The average values of 0.1% liver glycogen, of 434 (±52) mg. % muscle glycogen and of 90 mg. % blood sugar, when deducted from the corresponding values in Table I, permit a rough estimate to be made of the percentage of absorbed sugar that is accounted for by oxidation plus glycogen storage. In this calculation it is assumed that the muscles constitute 50% of the body weight and that the blood sugar is in equilibrium with 50% of the body weight. The percentages thus accounted for are entered in the last column of Table II; they are shown for comparative purposes only and are not meant to represent a balance for which a determination of glycogen deposition in the entire musculature and in tissues other than muscle would be required. Urinary excretion of sugar was determined in some animals but was found to be negligible.

‡ Some of these animals were injected with one cc. of active extract one hour before the sampling of the tissues with no apparent effect on the glycogen levels.

The blood sugar level and the O_2 consumption were higher in the rats injected with active extract than in the controls. The dose of insulin injected produced in both groups of animals only slight changes in carbohydrate oxidation and glycogen storage.

Summary. Rats fasted for 24 hours, when injected intraperitoneally with one cc. of anterior lobe extract shortly before glucose feeding, show a marked decrease in the amount of carbohydrate oxidized and a corresponding increase in the amount of glycogen deposited in liver and muscles, when compared with control rats treated exactly alike and injected with 1 cc. of heat-inactivated extract.

9226 P

Relative Effectiveness of Iodine in Thyroxin, Diiodotyrosine, and Potassium Iodide in Inducing Metamorphosis in Amphibia.*

Allen Lein. (Introduced by Bennet M. Allen.)

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It has been demonstrated repeatedly by several workers that the tadpole test for thyroid is an extremely sensitive one, and this method has been used extensively in determining the amount of thyroid-like activity in various substances. Using this test, Swingle,[1] in a series of publications, advanced evidence which indicated that iodine appeared to function independently as a hormone without the intermediation of the thyroid gland. This hypothesis received confirmation from the work of several additional experimenters. Among the iodine-containing compounds used in these tests, thyroxin and diiodotyrosine, due to their chemical similarity, were considered to be of particular significance. It was shown that thyroxin and diiodotyrosine produce the same physiological response qualitatively but that the former is much more active than the latter.[2, 3]

The present work was undertaken in order to obtain quantitative data on the relative effectiveness, *per unit of iodine,* of thyroxin,

* The writer wishes to express his gratitude to Prof. B. M. Allen and Mr. Boris Krichesky for suggesting this problem and for their many valuable suggestions throughout this work.

1 Swingle, W. W., *Endocrinol.*, 1918, **2**, 283; *J. Exp. Zool.*, 1919, **27**, 397, 417; *Science*, 1922, **56**, 720; Proc. Soc. Exp. Biol. and Med., 1926, **24**, 205.

2 Gaddum, J. H., *J. Physiol.*, 1927, **64**, 246.

3 Romeis, B., *Klin. Wochensch.*, 1922, **1**, 1262.

diiodotyrosine, and an inorganic iodine compound, potassium iodide. It was believed that such data would indicate the degree to which the action of iodine is dependent on the remainder of the molecule in which it is a constituent.

The test animals used in this work were 270 *Bufo halophilus* larvae which were selected so that there was a maximum variation of 3 mm. in body length and 5 mm. in total length; posterior limb buds measured 0.3 mm. or less. These animals were divided into 4 general groups: (1) controls, which were immersed in fresh, previously aerated tap water; (2) animals immersed in a thyroxin solution prepared to a concentration of 1 gm. thyroxin to 50,000,000 cc. of water (this solution was used as a standard throughout the experiment); (3) animals immersed in diiodotyrosine solutions prepared to have iodine concentrations ranging from that of the standard thyroxin solution to 400 times that of the standard thyroxin solution; (4) animals immersed in solutions of potassium iodide prepared to have iodine concentrations ranging from that of the standard thyroxin solution to 100 times that of the standard thyroxin solution. Extreme precaution was taken to avoid contamination and to maintain conditions as constant as possible. All solutions were made with previously aerated water; no food was given to the animals during the course of the experiment; and solutions were changed every 2 days throughout the 12-day period during which the animals were exposed to the iodine containing substances. Measurements of body length and total length of each animal were made at the beginning and end of the experiment, and measurements of the posterior limbs were made microscopically with the aid of an eye-piece micrometer.

Measurements indicated that, in accordance with the work of Allen,[4] extremely dilute solutions of thyroxin will induce precocious metamorphosis. The posterior limbs of the animals immersed in the standard thyroxin solution attained a length of 2.34 mm. as compared with 0.43 mm. in the controls. A diiodotyrosine solution having an iodine concentration equal to that of the standard thyroxin preparation had no measurable thyroxin-like activity. The most dilute solution of diiodotyrosine exerting a small but definite stimulus toward metamorphosis in the tadpoles was one having an iodine concentration 20 times greater than that present in the thyroxin used, and this stimulus toward metamorphosis increased with an increase in concentration of diiodotyrosine. Thus, tadpoles immersed in diiodotyrosine solutions having 300 times the amount of iodine

[4] Allen, B. M., *Anat. Rec.*, 1932, **54**, 45.

present in the standard thyroxin solution exhibited marked indications of metamorphosis which were comparable to those produced by the standard thyroxin solution. The posterior limbs of the animals immersed in this solution of diiodotyrosine grew to an average length of 2.04 mm. as compared with 2.34 mm. in the animals treated with thyroxin. Potassium iodide, in the concentrations used, showed no thyroxin-like properties. That this compound lacks this characteristic, however, is not a necessary conclusion for, as Swingle (1919) has indicated, still higher concentrations over greater periods of time may produce the desired effect. Experiments which may yield quantitative data on this question are now being planned in this laboratory. On the basis of the data now on hand, however, it may be said that iodine in diiodotyrosine is probably more than 5 times as active as that in potassium iodide.

Summary. Iodine as it occurs in thyroxin is over 300 times as effective in inducing precocious amphibian metamorphosis as that occurring in diiodotyrosine. The iodine in diiodotyrosine, however, is far more active than that included in potassium iodide.

9227 P

Utilization of Ketone Bodies by the Tissues in Ketosis.

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The work of Chaikoff and Soskin[1] and Mirsky[2] has shown very definitely that the ketone bodies are produced only in the liver. Are the ketone bodies so formed utilized by the tissues in nutritional conditions giving rise to ketosis? This note deals with this question.

A male human was kept on a ketogenic diet for several days. After a definite ketonuria had developed (5 gm. ketone bodies per day), food was withheld during the morning and blood ketone concentrations at 7 A. M. and 11 A. M. were determined (the first blood 12 hours after the previous meal). The 7 A. M. blood contained 26.6 mg. ketones %, the 11 A. M. sample 10.3. The experiment was repeated on 2 other mornings with the following results:

1. Blood ketones 7:20 A. M. 26.4, 11 A. M. 9.5.

1 Chaikoff, I. L., and Soskin, S., *Am. J. Physiol.*, 1928, **87**, 58.

2 Mirsky, A., *Am. J. Physiol.*, 1936, **116**, 110.

2. Blood ketones 7:50 A. M. 35.9, 12 Noon 8.8.

The results of Chaikoff and Soskin indicate that the ketone bodies diffuse very readily throughout the tissues of the body, so that we may assume that the subject (wt. 70 kilos) had at least 50 liters of water in his body carrying the ketones bodies in the same concentration as the blood. On this assumption the amount of ketones disappearing from the body during the mornings cited must have been (difference in concentrations of the blood ketones X 50 liters), 8.15 gm., 8.45 gm., 13.5 gm. The ketone output in the urines of these periods was 472 mg., 278 mg., 538 mg. This suggests a very considerable utilization of ketones by the tissues under a definitely ketogenic condition—averaging 2.44 gm. per hour, and does not include what might have been produced concurrently by the liver. All the foregoing estimations were carried out by the methods of Van Slyke (urine) and Van Slyke and Fitz (blood).

To obtain data bearing on the total tissue utilization arterio-venous ketone-body differences were determined in various conditions which give rise to definite ketosis. The estimations were carried out by the Barnes method.[3] The figures indicate the total ketone bodies expressed as mg. acetone per 100 cc. blood.

TABLE I.

Species	Condition	Arterial Blood	Venous Blood
Man	Ketogenic diet 2 days, fasting 20 hr.	3.3	2.4
,,	,, ,, ,, ,, ,, ,,	4.4	2.7
,,	,, ,, 3 ,, ,, ,, ,,	17.3	14.1
Dog	2 days fast and phloridzin	3.2	1.9 (Femoral)
			2.5 (Jugular)
,,	4 ,, ,, ,, ,,	19.3	17.7 (Femoral)
			17.7 (Jugular)
,,	,, ,, ,, ,, ,,	14.0	11.8
,,	Depancreatized without insulin 30 hr.	3.5	2.9
,,	,, ,, ,, ,, ,,	3.7	3.5
Rabbit	Fasting and injection of ketogenic extract of ant. pituitary	7.2	6.5

The differences are significant, since for man an arterio-venous difference of 1 mg. % and a tissue blood flow of 5 liters per min. would indicate a utilization of 3 gm. ketones per hour. This is of the same order of magnitude as the figure given above. The oxidation of such an amount would give rise to considerable energy and would represent an appreciable fraction of the total metabolism. We may conclude then that the mechanism: conversion of fats to ketones by the liver and oxidation of this by the tissues, may account for an

[3] Barnes, R. H., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 352.

important amount of the metabolism of individuals having "ketosis". It follows that this mechanism of fat utilization could operate when the body is oxidizing fat without showing ketosis.

9228 P

A Method for Determination of Blood Acetone Bodies.

RICHARD H. BARNES. (Introduced by Eaton M. MacKay.)
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A satisfactory method for the determination of the acetone bodies in blood at low levels or suitable for use with small volumes of blood has not hitherto been available. An attempt has been made to devise a method which combines the sensitivity of the iodine titration (Hubbard's Method) with the specificity of the Denigè's-Van Slyke method. In this method the ketone bodies are precipitated as in Van Slyke's method, this precipitate is freed from contaminating material by washing, it is dissolved in acid and is distilled with heat into alkaline iodine which can be titrated with thiosulfate. Oxidation of the ketones is carried out in a large centrifuge tube with a small volume of liquid, which allows the acetone precipitate to form with very small quantities of acetone. Barium chloride is added and the fine barium sulfate precipitate helps to prevent the loss of the acetone mercuric sulfate precipitate when decanting.

Briefly the procedure is as follows: 2 cc. of oxalated blood is lyzed with 14 cc. water in a 50 cc. centrifuge tube and 14 cc. of mercuric sulfate solution (as in the Van Slyke method[1]) added with shaking. After standing an hour this is centrifuged and the supernatant liquid filtered. Ten cc. of filtrate* is placed in a special centrifuge tube with a ground glass joint and 4 cc. of a solution added which contains per 100 cc.: 70 cc. of the above mentioned mercuric sulfate solution, 20 cc. of 50% sulfuric acid, and 10 cc. of water. Connected by the glass joint to a reflux condenser this mixture is boiled for 90 minutes. As soon as boiling has commenced 0.5 cc. of 5% potassium dichromate is added. The tube is then cooled, 3-4 drops of 10% barium chloride is added and then centrifuged at high speed for 10 minutes and the supernatant liquid carefully poured off.

[1] Peters and Van Slyke, *Quantitative Clinical Chemistry*, 625.

* Filtrates prepared in this manner from diabetic blood must be treated further for the high sugar present.

TABLE I.
Recovery of Acetone from Pure Acetone Solutions and Following the Oxidation of β-hydroxy-butyric Acid (Racemic).

Acetone		β-hydroxy-butyric acid	
Present mg.	% recovered	Mg. % by Van Slyke method	% recovered
.0178	71.4	3.96	75.8
.0378	73.6	11.75	73.1
.0550	74.6	24.1	79.3
.0735	70.1	36.1	77.0
.0770	71.2	59.1	77.9
.0910	73.6		
.0960	76.0		
.1098	74.5		
.1543	76.6		
.1704	75.0		
.1931	74.9		
Aver.	73.9		76.6

Five cc. of 10% sodium hydroxide is used to wash the precipitate and decanted after centrifuging. Ten cc. of 20% hydrochloric acid is added and the tube connected by a ground glass joint to a distilling condenser, the delivery tip of which dips below the surface of an excess of iodine (.001 N) made alkaline with 5 cc. of 40% sodium hydroxide. After distillation of almost all the liquid in the special tube, the receiving flask solution is acidified with 50% sulfuric acid and the excess iodine titrated with thiosulfate. In Table I is shown the recovery of acetone from pure solutions (74%) and from the Denigè's-Van Slyke precipitate formed by the oxidation of β-hydroxy-butyric acid (76%). Recovery of these substances when added to ketone-free blood is essentially the same. Until more information as to the relative concentrations of the various acetone bodies in the blood in ketonemia is available we are expressing our results as acetone, and using the factor 1.33.

9229

Early Effect of Total Thyroidectomy in a Case of Polycythemia vera (Vaquez-Osler Syndrome).

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Up to the present time the etiology of polycythemia vera has not been clarified nor have the methods of treatment proposed met

with lasting success. It is generally agreed (Vaquez[1] and Osler[2]) that the hematopoietic tissues are primarily at fault. One group of workers look upon erythremia as a neoplasm of the bone marrow (Minot and Buckman[3]), another as an excessive response to agents formed in the liver, stomach (Marshall[4] and Morris[5]), and endocrine organs (Gunther,[6] Moehlig and Bates,[7] and others), and a third group visualizes the disease as an anoxemic response induced by subintimal and advential fibrosis in the arteries and arterioles of the bone marrow (Reznikoff, Foot and Bethea[8]).

In polycythemia the patho-physiological changes in the peripheral blood are characterized by an increase in the number of red blood cells per cmm., an increase in the hemoglobin, an increase in the volume of packed cells with a corresponding decrease in plasma volume (hematocrit reading) and an increase in the total circulating blood volume.

When one searches for a means of correcting this anomaly, the state of myxoedema presents itself. It has been shown (Minot,[9] Emery,[10] Mackenzie,[11] and Stone[12]) that anemia is a frequent if not constant finding in myxoedema; and there is abundant evidence (Tatum,[13] Lim,[14] and Kunde[15]) to indicate that the abolition of thyroid function would depress erythropoiesis. Myxoedema is also associated with a decreased blood volume (Thompson,[16] Goldbloom and Libin[17]). It seemed, therefore, a rational therapeutic procedure to subject a patient with polycythemia vera to a total thyroidectomy. This has been done and the results are here reported.

The patient's complaints were those usually encountered in this

1 Vaquez, M. H., *Comp. rend. Soc. de biol.*, 1892, **44**, 384.

2 Osler, William, *Am. J. Med. Sc.*, 1903, **126**, 187.

3 Minot, George R., Buchman, Thomas E., *Am. J. Med. Sc.*, 1923, **166**, 469.

4 Marshall, L. H., *Am. J. Physiol.*, 1935, **114**, 194.

5 Morris, R. S., Schiff, L., and Foulger, M., *J. Med.*, 1932, **13**, 318.

6 Gunther, H., *Endocrinology*, 1930, **14**, 184.

7 Moehlig, R. C., and Bates, G. S., *Arch. Int. Med.*, 1933, **51**, 207.

8 Reznikoff, Paul, Foot, Nathan C., and Bethea, James M., *Am. J. Med. Sc.*, 1935, **189**, 753.

9 Minot, George R., *Med. Clinics North America*, 1921, **4**, 1733.

10 Emery, Jr., E. S., *Am. J. Med. Sc.*, 1923, **165**, 577.

11 Mackenzie, G. M., *J. A. M. A.*, 1926, **136**, 462.

12 Stone, C. T., *Ann. Int. Med.*, 1928, **11**, 215.

13 Tatum, A. L., *J. Exp. Med.*, 1913, **17**, 636.

14 Lim, R. K. S., Sarhar, B. B., and Jane, P. H., Brown, Grahem, *J. Path. and Bact.*, 1922, **25**, 228.

15 Kunde, M. M., *Am. J. Physiol.*, 1932, **99**, 469.

16 Thompson, W. O., *J. Clinical Invest.*, 1925-26, **2**, 477.

17 Goldbloom, A. Allen, Libin, Isaiah, *Arch. Int. Med.*, 1935, **55**, 484.

disease, headache, dizziness, fullness in the head, flushing of the face with cyanosis of lips, hands and feet, weakness, dyspnoea, nausea and occasional attacks of vomiting. The appearance of the patient, the engorgement of retinal veins, enlargement of the spleen, and the hematological studies completed the diagnosis.

The oxygen consumption values prior to thyroidectomy, as determined by breathing oxygen (Benedict-Roth apparatus) on 4 different days were plus 23, 22, 33, and 25%. On 3 other days using the Tissot gasometer and the gas analysis method values of plus 26, 22, and 35% were obtained. The cholesterol was 116 mg. %. Fifteen months after thyroidectomy the oxygen consumption gradually fell until it reached a value of minus 30%.

At this time the blood cholesterol was 250 mg. % and the clinical picture that of myxoedema.

The hematological changes which have occurred in the fifteen months period may be summarized as follows:

The hemoglobin values (gm. per 100 cc.) prior to thyroidectomy were 21, 21.5, and 19.0; and afterwards 20, 19, 19. The erythrocytes decreased from 8.25, 8.62 and 8.89 (millions) to 6.58, 6.32, and 6.50 (millions). The mean corpuscular volume values (Wintrobe) of 89, 92, 85.4 have not been changed significantly, but remain (94.2, 98.1, 95.3) at the upper limit of normal.

The mean corpuscular hemoglobin (mmgr.) has increased from a somewhat lower than normal value of 25.5, 24.8, 21.3 to a normal value of 30.4, 30.0, 29.2. With a reduction in the number of red cells there has occurred a return to normal of the hemoglobin load of the individual cells.

The mean corpuscular hemoglobin concentration, which is a function of the volume of packed cells has increased from 27.6, 26.1, 25.0% to 32.3, 30.0, 30.5%, but remains below the normal value, 35%. The white blood, the differential, and the reticulocyte counts have not been altered.

The volume changes have been most interesting and significant. The total plasma volume of 1927 cc. (1114 cc. per sq. meter surface) has increased to 2436 cc. (1350 cc. per sq. m.). If this is compared with an average normal value of 2090 cc. (1303 cc. per sq. m.) obtained in the case of 6 females in our laboratory, it will be seen that the plasma volume (per sq. m. surface) prior to thyroidectomy was somewhat below normal, but that it has now reached normal.

The hematocrit has dropped from 82% to 62%, but it has not as yet reached normal (44%). The blood volume has decreased

from 10706 cc. (6187 cc. per sq. m.) to 6447 cc. (3581 cc. per sq. m.). Since this is a function of the hematocrit the values would remain elevated until the hematocrit becomes normal.

The total circulating hemoglobin of 2194.73 gm. has been reduced to 1224.96 gm. This visualizes more clearly the effect of total thyroidectomy on erythropoiesis and hemoglobin formation than do hemoglobin concentration studies. It is probable that the patient's relief from symptoms is attributable to this reduction.

Conclusions. (1) All the alterations in the blood following a total thyroidectomy in a case of polycythemia vera are in the direction of normal. Normal values for total blood plasma, mean corpuscular hemoglobin (γ, γ) and mean corpuscular hemoglobin concentration have been attained. Although the concentration of hemoglobin per 100 cc. has remained unchanged the total circulating hemoglobin has been reduced to 56%. The other changes (hematocrit, total blood volume) are so striking as to be significant.

(2) The patient has been relieved of her former symptoms. Her present complaints are due solely to the myxoedema. No attempt has been made to control these as this would defeat the experimental objective.*

9230

Studies Relating to Time of Human Ovulation. II. During Lactation.

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It is a well known clinical fact that in the absence of any contraceptive measures, some women will become pregnant during their period of lactation while others will not. This has led to the conclusion that ovulation occurs in the former group but not in the latter. The frequency of ovulation in lactating women who are having catamenia at regular intervals is at present unknown. The following study was undertaken in order to shed some light on the subject.

A group of patients reporting to the postpartum clinic of the

* The authors wish to thank Doctors Ford K. Hick and M. H. Streicher for technical assistance in completing some of these studies.

Sloane Hospital was chosen. Each patient was nursing regularly. Endometrial biopsies were taken at 4-week intervals by means of a modified Klingler and Burch[1] suction curette (Kurzrok[2]). On the basis of this biopsy a diagnosis was made of the phase of the menstrual cycle. The presence of a premenstrual endometrium implies that ovulation has taken place and that we are dealing with an ovulatory cycle. On the contrary, the demonstration of a proliferative endometrium within 10 days of the succeeding flow implies an absence of ovulation or an anovulatory cycle. One of us (R. K.) has previously shown[3] that the limits of ovulation during a regular 28-day cycle lie between the 9th and the 21st day of the cycle, with the greatest probability that ovulation will occur between the 12th and 14th day. The first day of bleeding records the first day of the cycle.

Thirty cases were chosen and more than 125 biopsies have been obtained. Of these one case had 9 consecutive monthly biopsies, one case had 8, 2 had 7, 2 had 6, 5 had 5, while 3 cases had 4, 9 had had 3, and the 7 remaining cases have had at least 2 biopsies taken. The spotting that occasionally resulted from a biopsy never lasted more than a few hours. (We have taken to date more than 1500 biopsies with only one slight accident.)

In Table I the diagnosis of the menstrual phase was made for each biopsy; the figure to the left gives the number of days since the previous flow (to the time the biopsy is taken), and the figure to the right the number of days before the next period. Thus, 27) P (1, means a biopsy was taken 27 days after the last flow and one day before the next flow. A proliferative endometrium 1 to 10 days before the flow implies an absence of a corpus luteum, hence an absence of ovulation, therefore an anovulatory cycle. Two patients (6 and 28) stopped having periods while they were under observation. Thus 86) P means that a proliferative endometrium was found on the 86th day since the last period of bleeding, no catamenia having occurred during this time.

Conclusions. Thirty lactating women studied during their postpartum period had 106 fairly regular cycles. Thirty-nine cycles were ovulatory in character, hence fertile. Forty-five cycles were anovulatory or sterile, hence the bleeding at the end of each cycle was not true menstruation for it did not come from a premenstrual

1 Klingler, H. J., and Burch, J. C., *J. A. M. A.*, 1932, **99**, 559.

2 Wilson, L., and Kurzrok, R., *Am. J. Obs. and Gyn.*, 1936, **31**, 911.

3 Kurzrok, R., Kirkman, I., and Creelman, M., *Am. J. Obs. and Gyn.*, 1934, **28**, 319.

TABLE I.

Case No.	Age	Days between delivery and first post-partum period	Biopsy No. 1	Biopsy No. 2	Biopsy No. 3	Biopsy No. 4	Biopsy No. 5	Biopsy No. 6	Biopsy No. 7	Biopsy No. 8	Biopsy No. 9
1	34	29	27) P (1	23) P (59	84) P (3	27) P (1	28) P (2	25) P (7	20) S (8	20) P *	
2	27	76	21) P (2	27) P (1	28) P (2	27) P (8	18) P (7	20) P (11	20) P (2 *		
3	26	60	53) P (10	80) P (16	11) P (17	12) P (14	39) P (4				
4	35	91	21) P (1	28) S (26	23) S (2 *	26) P (3	24) S (7	22) S (3			
5	23	63	25) P (15	20) S (1 *	29) S (1						
6	32	53	19) P (45	49) P (11	16) P (6	28) P (7	22) P *	56) P	86) P	124) P (8	24) S (3
7	26	44	21) P (102	82) C.G.H. (13	24) P (13						
8	25	47	25) S (4	17) C.G.H. (13	26) S (3						
9	37	57	27) S (4	24) S (4							
10	20	102	30) S (1	27) S (1	28) S (1 *						
11	25	51	25) P (3	46) P (2							
12	28	50	9) P (1	28) S (1 *							
13	22	47	23) P (8	34) S (1	35) S (1	29) S (1	28) S (2				
14	24	42	30) P (58	26) P (6							
15	28	40	24) P (1	28) P (1	28) P (31	35) P (3	30) S				
16	25	42	7) P (15	20) P (1	27) P (1	14) S (1	28) P (1				
17	18	127	24) P (3	26) P (5	22) P (10						
18	25	34	23) P (1	51) P (1	23) P (1	17) P (1	13) Early S(1	18) Early S(18	21) P (9		
19	28	130	29) P (8	33) P (1 *	18) P						
20	28	69	22) P (6	22) S (7 *	32) Early S(4						
21	22	100	51) P (22	20) S (7	21) P (12	23) P (11	28) P (1				
22	27	101	27) P (1	29) P (11	21) P (12	23) P					
23	27	120	20) P (8	23) S (2	26) S						
24	27	74	24) P (13	36) S *	87) Decidua	115) Decidua					
25	27	20	21) Atypical S(1	27) S (1							
26	22	84	29) Early S(2	12) P (16							
27	27	80	26) S (3	23) S (7	49) S (1	27) S					
28	22	42	22) P *	51) P	79) P	107) P	142) P	240) P			
29	25	42	5) P (30	26) Early S(8	20) S (7						
30	26	66	28) P (14	15) P (10							

P = Proliferative or Postmenstrual Endometrium.
S = Secretory or Premenstrual Endometrium.
C.G.H. = Cystic Glandular Hyperplasia of the Endometrium.
* = Weaning.
Note: Neither the parity of the patient, nor the nursing schedule are in any way correlated with the result.

endometrium. There were 22 cycles of doubtful interpretation, by which we mean that 10 or more days elapsed between the date of the biopsy and the onset of the next period of bleeding. Ovulation could, therefore, potentially occur between the date of biopsy and the onset of the flow. Forty-two to 63% of the lactating women who bleed at fairly regular intervals have anovulatory or sterile cycles.

9231

Crystallization of Liver Fraction Protecting Against Necrosis from Carbon Tetrachloride or Chloroform Administration.

J. C. FORBES AND JEANETTE S. MCCONNELL. (Introduced by H. B. Haag.)

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We have reported[1, 2] on the use of a liver preparation in the prevention of liver necrosis from carbon tetrachloride or chloroform administration. At this time we wish to report a method for the preparation of this active principle in a crystalline form.

A concentrated aqueous extract of hog liver representing approximately 10 gm. per cc., from which the heat coagulable materials have been removed, is warmed in a water bath to a temperature of approximately 60°C. To each 1000 cc. of this solution 2400 cc. of ethyl alcohol of about the same temperature is added with stirring. The precipitate which forms is filtered off after cooling and 2000 cc. of a saturated aqueous solution of ammonium sulfate added to the filtrate. The solution is then thoroughly shaken. On standing it separates into 2 layers: above, an alcoholic layer and below, a watery layer containing a great deal of precipitated ammonium sulfate. The upper layer is syphoned off and 1500 cc. of alcohol added to it to precipitate excess ammonium sulfate. The solution is filtered after being cooled in a refrigerator for several hours. The filtrate is then evaporated under reduced pressure to approximately 170 cc. It is then placed in a refrigerator and cooled over night. The precipitate is separated by centrifuging and washed in the centrifuge tubes, first with about 60 cc. of ice cold water and then

1 Forbes, J. C., and Neale, R. C., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 319.

2 Forbes, J. C., Neale, R. C., and Scherer, J. H., *J. Pharm. and Exp. Therap.*, 1936, **58**, 402.

with approximately 40 cc. of cold alcohol. The residue is suspended in 40 to 60 cc. water, depending upon the amount present, and concentrated sodium hydroxide added drop by drop with thorough mixing until needle-like crystals separate out or form when a drop of the solution is placed on a microscope slide and allowed to evaporate slightly. As a rule a satisfactory pH is approximately 9.3. After the correct amount of alkali is added the solution is set aside in a refrigerator for a number of hours and then filtered with suction. The crystalline material may be recrystallized from warm water in which it is soluble to the extent of about 30 mg. per cc. Hot water converts it slowly into an insoluble form.

The pure crystalline material is almost snow white. It gives a strong murexide test. It is precipitated by silver nitrate, picric acid, metaphosphoric acid, phenol and tricresol. Its chromogenic activity with Folin's uric acid reagent is approximately 1/350 that of uric acid. The nitrogen content of a sample recrystallized from water several times and dried at 125°C. for 24 hours was found to be 27.42%. These properties indicate that the substance is a purine derivative but apparently different from any previously reported.

The crystalline material when injected subcutaneously in doses of 100 mg. per 100 gm. of rat weight affords excellent protection against carbon tetrachloride or chloroform poisoning. Owing to its relatively low solubility in water we usually administer it, partly in solution and partly in suspension in concentrations of 50 mg. per cc. Also, in order to afford ample time for absorption, the animals are protected as a rule 18 to 24 hours prior to the time of acute poisoning. This crystalline material has also been used quite successfully in the prevention of cirrhosis from chronic carbon tetrachloride poisoning.

The nitrogen value was kindly supplied by Dr. R. C. Neale, Biochemical Research Foundation, Philadelphia, who is studying the chemical structure of the material. Frozen livers were used altogether in this investigation.

9232

Effect of Adrenal Cortical Hormone on Renal Excretion of Electrolytes in Normal Subjects.

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(With the technical assistance of Irving Miller.)

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The effect of the adrenal cortical hormone on the renal excretion of electrolytes in normal human subjects has been described.[1] A marked alteration in the renal excretion of sodium and potassium was noted during a 5-hour period in which the subjects received adrenal cortical hormone intravenously; little or no effect was observed on the subsequent 24-hour excretion. Subcutaneous injections of hormone in a quantity sufficient to produce a change in the sodium and potassium balance of patients with Addison's disease did not affect the balance of electrolytes in normal subjects. It seemed probable that repeated injections of larger quantities of hormone might produce a significant alteration in the 24-hour renal excretion.

In the present study the effect of repeated intravenous injections of hormone† on the 24-hour renal excretion of 3 normal subjects has been observed.

Three subjects (F. D., female, 14 years of age; D. W., female, 29 years of age; G. W., male, 31 years of age) were provided with a diet of constant mineral composition and a constant fluid intake. The subjects had been maintained on a constant metabolic regime for other purposes during a period of 3 to 4 weeks immediately preceding this investigation.

The 24-hour urine collection was completed at 7 A. M. The subjects were injected intravenously with adrenal cortical hormone (500 dog units) at 8 A. M., 12 M., 6 P. M., and 11 P. M. An amount of normal saline solution equivalent to the sodium chloride content of the extract was injected during the 24-hour control period.

The preparation of the diet and the methods used for determining the sodium, chloride, phosphate and total nitrogen content

* Rockefeller Fellow in Medicine.

1 Thorn, G. W., Garbutt, H. R., Hitchcock, F. A., and Hartman, F. A., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 247.

†Adrenal cortical hormone used in this study was generously supplied by Doctor David Klein of the Wilson Laboratories, Chicago, Illinois, and by Doctor George F. Cartland of the Upjohn Company, Kalamazoo, Michigan.

of both diet and urine specimens have been described.[2] Potassium determinations were made according to the method of Shohl and Bennett[3] on specimens ashed with thorium nitrate.[4] Calcium was analyzed according to the method of Tisdall and Kramer.[5] The method of Briggs[6] was used for the determination of magnesium. Total base was calculated as the sum of the individual cations, determined separately.

The injection of adrenal cortical hormone resulted in a marked decrease in the excretion of sodium in all subjects. Diminished urine volume and chloride excretion were associated with the reduced output of sodium. During the period of hormone administration the concentration of sodium in the urine was actually reduced; the concentration of chloride was not altered significantly. In contrast to the decreased sodium, chloride and urine output, the excretion of potassium was increased during the period of hormone injection. No consistent alteration occurred in the renal excretion of either calcium or magnesium.

The total base content of the urine decreased during the period of hormone injection. As a result of the diminished urine volume and the increase in the excretion of potassium, however, the concentration of total base was increased.

The excretion of inorganic phosphate was increased in 2 subjects during the period of hormone administration. Total nitrogen excretion was not altered significantly.

On the day following injection of the hormone, excretion of sodium was greatly increased in all subjects. Potassium excretion was reduced during the same period. This complete alteration in the sodium and potassium excretion on the day following hormone injection has been observed to occur in normal dogs injected with adrenal cortical hormone.[7] The change might be described as a "rebound" phenomenon.

The injection of hormone was accompanied by a slight gain in body weight (0.1 to 0.3 kg.) in all subjects. A comparable weight loss occurred on the day subsequent to hormone administration. The hormone had no demonstrable effect on the loss of insensible water‡

2 Thorn, G. W., Garbutt, H. R., Hitchcock, F. A., and Hartman, F. A., *Endocrinology*, 1937, **21**, 202, 213.

3 Shohl, A. T., and Bennett, H. B., *J. Biol. Chem.*, 1928, **78**, 643.

4 Strauss, M., *J. Biol. Chem.*, 1937, **118**, 331.

5 Tisdall, F. F., and Kramer, B. J., *J. Biol. Chem.*, 1921, **48**, 1.

6 Briggs, A. P., *J. Biol. Chem.*, 1924, **59**, 255.

7 Harrop, G. A., and Thorn, G. W., *J. Exp. Med.*, 1937. (In press.)

‡ The loss of insensible water has been approximated by measuring the reduction in body weight which occurred during a 10-hour period (9 P.M. to 7 A.M.), correction being made for the weight of ingesta and excreta.

TABLE I.

Effect of Repeated Intravenous Injections of Adrenal Cortical Hormone* on the Twenty-four Hour Renal Excretion of Electrolytes in 3 Normal Subjects.

Subjects and Body wt., kg.	Urine vol., cc.	Na, m. eq.	K, m. eq.	Ca, m. eq.	Mg, m. eq.	Total base, m. eq.	Conc. of base, m. eq./liter	Chloride, m. eq.	Inorganic phosphate, mg.	Total N, gm.	Insensible water loss, gm./hour	Mean atmospheric temp., °F.	Relative humidity, % noon	Day	Injection
F. D.															
39.8	1720	94.6	56.6	4.7	2.4	158.3	92.0	110.0	679	6.79	30	34	47	1	Control Saline
39.9	1240	52.9	59.0	4.6	1.8	118.3	95.4	76.4	638	6.38	30	28	44	2	Hormone
39.6	1540	103.5	47.8	5.5	2.2	159.0	103.2	99.5	677	6.77	40	42	31	3	Control Saline
D. W.															
50.5	1360	109.0	48.8	1.8	0.9	160.5	118.0	108.0	588	7.46		46	59	1	Control Saline
50.7	1150	85.8	57.0	1.5	0.9	145.2	126.2	102.5	614	7.56		35	48	2	Hormone
50.6	1150	103.0	29.8	1.6	0.9	135.3	117.6	92.0	495	6.90		40	92	3	Control Saline
G. W.															
75.2	590	83.5	48.5	1.0	0.4	133.4	226.1	85.2	635	7.84		41	72	1	Control Saline
75.5	500	64.9	59.4	2.1	0.4	126.8	253.6	72.6	735	7.66		46	59	2	Hormone
75.2	700	122.0	31.6	2.5	0.5	156.6	223.7	112.0	592	8.06		35	48	3	Control Saline

The values expressed for urine volume, sodium, potassium, calcium, magnesium, chloride, phosphate, total base, and total nitrogen refer to the total 24-hour renal excretion.

*Adrenal Cortical Hormone (500 dog units) was injected intravenously at 8 A.M., 12 M., 6 P.M., and 11 P.M.

in subject F. D. (Table I). The greater loss which was observed during the control period following hormone injection was within previously noted daily variation.

No untoward reaction has been observed during the intravenous injection of quantities of hormone as large as 30 cc. (3,000 dog units). In experimental studies on human subjects the hormone has been injected intravenously since the subcutaneous injection of large quantities of extract is painful. The transitory action of the hormone following its intravenous administration necessitates repeated injections throughout the 24 hours to produce a continued effect.

It is particularly significant that sodium was the only substance studied which was reduced both in total amount and concentration during the period of hormone injection. Chloride excretion was reduced but the concentration of chloride in the urine was not altered significantly. The injection of hormone was associated with an increased excretion of potassium. The increase in potassium excretion in the normal subjects was not nearly so great as that noted in patients with Addison's disease.[2] It is probable that in Addison's disease other factors such as loss of base from extra-cellular compartments, increased water content of cells, destruction of cells and nitrogen retention, may modify the action of the adrenal cortical hormone.[8]

Summary. Repeated intravenous injections of adrenal cortical hormone under controlled conditions in 3 normal subjects resulted in a decreased renal excretion of sodium, chloride and water. During the same period inorganic phosphate excretion was increased in 2 of the subjects. The excretion of calcium, magnesium and total nitrogen was not significantly affected. The retention of sodium, chloride and water was reflected in a slight increase in body weight in all of the subjects. Withdrawal of hormone was accompanied by a marked increase in the excretion of sodium, a reduction in potassium excretion, and a loss of weight. Repeated intravenous injections of adequate quantities of adrenal cortical hormone, therefore, appear to influence the 24-hour renal excretion of electrolytes in normal subjects. The nature of the change produced by the hormone is similar to that which has been noted in patients with Addison's disease but is quantitatively less marked.§

8 Harrop, G. A., Nicholson, W. M., Soffer, L. J., and Strauss, M., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1312.

§ The author is indebted to Doctor George A. Harrop for his helpful suggestions and stimulating criticism.

9233

Phosphorus Components in the Blood of Normal and Rachitic Infants.

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The inorganic phosphorus of the blood in rickets has been studied at great length. In recent years comparable attempts[1-5] have been made to determine the acid soluble organic phosphorus compounds. These investigations have been handicapped by the fact that only adenosinetriphosphate[6] and diphospho-l-glyceric acid[7] have been isolated from blood and that such isolations do not as yet permit a quantitative estimation to be based on them. Consequently, indirect methods for the estimation of the organic phosphorus compounds in blood have been attempted, based upon the comparison of the rate and extent of the acid and phosphatase hydrolysis of blood filtrates and of such compounds as have been shown or are believed to exist in the blood. In view of the growing evidence, especially that recently submitted by Kerr and Daoud[8] and by Warweg and Stearns,[9] that organic phosphorus entities, calculated on the basis of such work, probably represent definite organic phosphorus compounds, a series of values are presented for groups of normal and rachitic children.

1. Fraction hydrolysable in 1 N acid (sulphuric or hydrochloric) in 10 minutes at 100°. Under these conditions, diphospho-l-glycerate and hexosediphosphate are hydrolyzed to a negligible extent.[9] Adenosine triphosphate from muscle[10, 11] or rabbit's blood[6] yields

1 Zucker, T. F., and Gutman, M., PROC. SOC. EXP. BIOL. AND MED., 1923, **20**, 372.

2 Kay, H. D., and Robison, R., *Biochem. J.*, 1924, **18**, 755.

3 Barrenscheen, H. K., and Vasarhelyi, B., *Biochem. Z.*, 1931, **230**, 330.

4 Bakwin, H., Bodansky, O., and Turner, R., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1238.

5 Stearns, E., and Warweg, G., *Am. J. Dis. Child.*, 1935, **49**, 79.

6 Fiske, C. H., *Proc. Nat. Acad. Sci.*, 1934, **29**, 25.

7 Greenwald, I., *J. Biol. Chem.*, 1925, **63**, 339.

8 Kerr, S. E., and Daoud, L., *J. Biol. Chem.*, 1935, **109**, 301.

9 Warweg, G., and Stearns, E., *J. Biol. Chem.*, 1936, **115**, 567.

10 Fiske, C. H., and Subbarow, Y., *Science*, 1929, **70**, 381.

11 Unpublished data. The specimen of adenosinetriphosphate was kindly supplied by Dr. Cyrus H. Fiske.

two-thirds of its phosphorus. The probability is that the phosphorus liberated from a trichloracetic acid filtrate represents two-thirds of the adenosine triphosphate phosphorus present.

2. Fraction not hydrolysable by bone phosphatase at pH of about 9.0 in a 4% concentration of trichloracetic ion. Under these conditions bone phosphatase does not hydrolyse diphospho-l-glycerate[12] but hydrolyses adenosinetriphosphate and sodium hexosediphosphate, the former to about 80%, the latter completely.[11] In addition, the concentration of this non-hydrolysable fraction parallels the amounts of diphospho-l-glycerate which Greenwald[7] obtained from different species. The phosphorus not liberated under these conditions probably represents, therefore, to within about 5% of the absolute value, the phosphorus from diphospho-l-glyceric acid.

Blood specimens were drawn between 3 and 5 hours after the morning meal. One volume of blood was added without the addition of an anticoagulant to 4 volumes of 10% trichloracetic acid and filtered within 10 minutes. Inorganic phosphorus, total and acid soluble phosphorus were determined by the method of Fiske and Subbarow. The readily acid hydrolysable phosphorus was determined by adding 2 cc. of trichloracetic acid filtrate to 2 cc.. of 2 N HCl, heating in a water bath at 100° for 10 minutes,[3, 4] and noting the inorganic phosphorus liberated. Values for concentration in the red blood cells were calculated.

Fraction Hydrolysable by Bone Phosphatase. To 5 cc. of the trichloracetic acid filtrate 2 cc. of 1 N NaOH and 2 cc. of 10% sodium diethylbarbiturate were added. One cc. of phosphatase extract[13] was added and the pH adjusted to 8.8 to 9.0. The mixtures were allowed to stand at room temperature with toluene until equilibrium had been reached as indicated by the constancy of the inorganic phosphorus readings (1 to 2 weeks).

The findings in normal and rachitic children are compared in Table I. The acid insoluble phosphorus is unchanged. In infants with rickets the acid soluble phosphorus is reduced. The inorganic phosphate, the readily acid hydrolysable phosphorus,[4] and the component not hydrolysable by bone phosphatase all participate in this reduction. The differences between the 2 groups for both whole blood and red blood cells are statistically significant.

The mean value for the readily acid hydrolysable phosphorus obtained in this study (4.9 mg. P per 100 cc. for normal infants) is in the same range as the values obtained in adults by Barrenscheen and Vasarhelyi (5.7 mg. per 100 cc.) and Kerr and Daoud

12 Bodansky, O., and Bakwin, H., *J. Biol. Chem.*, 1934, **104**, 747.

13 Bakwin, H., and Bodansky, O., *J. Biol. Chem.*, 1933, **101**, 641.

TABLE I.
Phosphorus Components in the Whole Blood and Red Blood Cells of Normal and Rachitic Infants.

	Whole Blood						Red Blood Cells					
	Non-Rachitic			Rachitic			Non-Rachitic			Rachitic		
	No. of cases	Mean and P.E.* mg. %	Standard Deviation mg. %	No. of cases	Mean and P.E.* mg. %	S.D. mg. %	No. of cases	Mean and P.E.* mg. %	Standard Deviation mg. %	No. of cases	Mean and P.E.* mg. %	S.D. mg. %
Total Phosphorus	41	38.8±.58	5.5	32	34.6±.42	3.5	—					
Acid Insoluble	41	12.5	—	32	12.2	—	—					
'' Soluble	56	26.3±.43	4.8	35	22.4±.32	2.8	35	67.5±1.18	10.3	30	60.0±1.01	8.2
Inorganic†	56	4.4±.06	0.7	37	2.5±.10	0.9	34	2.4±0.15	1.3	32	0.9±0.13	1.1
Organic	54	22.1±.28	3.0	34	20.0±.30	2.6	35	64.7±1.11	9.7	34	58.4±0.77	6.6
Readily Acid Hydrolysable†	55	4.9±.07	0.8	36	4.0±.09	0.8	45	14.0±0.28	2.8	36	11.8±0.24	2.1
Not Hydrolysable by Bone Phosphatase	36	14.0±.31	2.8	26	11.9±.35	2.6	35	40.6±1.06	9.3	26	33.9±0.89	6.7
Hematocrit %	47	35.0±.58	5.9	36	34.0±.46	4.1						
		Serum Inorganic Phosphate										
	39	5.3±.09	0.8	33	3.2±.12	1.0						

*P.E. means probable error.
†These groups include values previously reported.[4]

(5.4 mg. P per 100 cc.). Warweg and Stearns obtained a mean value of 2.4 mg. P for this fraction.

The fraction not hydrolysable by bone phosphatase, probably diphosphoglycerate, is 63.4% of the ester phosphorus in the normal and 64.6% in the rachitic group; these values agree well with the "phosphoglycerate" fraction as determined by Warweg and Stearns (mean, 68% of the ester phosphorus in adult man).

The residual phosphorus, after the readily acid hydrolysable and the "phosphoglycerate" phosphorus are subtracted from the ester phosphorus, is considered by Warweg and Stearns to represent phosphorus of hexosephosphate nature. Their estimate for this fraction is 20.6% of the ester phosphorus. They do not consider Fiske's isolation of adenosinetriphosphate from rabbit's blood and his findings that two-thirds of this phosphorus is readily hydrolysed. Kerr and Daoud[8] found that the ratio of the readily hydrolysable phosphorus to the nucleotide N was the same in the trichloracetic acid filtrate of human blood as in adenosinetriphosphate, indicating that the latter compound was present. Since the value given by Warweg and Stearns for the readily acid hydrolysable phosphorus seems too low, and since they do not consider the probability that it is a portion of the adenosinetriphosphate molecule, it would appear that their estimate for the hexosephosphate fraction is too high. According to our calculations, the residual phosphorus, probably hexosephosphate, is about 5% of the ester phosphorus; this value is much more in accord with the figure of Kerr and Daoud and the estimates of Mai[14] and Lawaczek.[15]

Summary. 1. The phosphorus of the blood was fractionated by a combination of acid and phosphatase hydrolysis and the results compared in normal and rachitic infants. 2. In infants with rickets there is a reduction in the acid soluble phosphorus of the blood which is made up of decreases in the inorganic phosphate, the readily acid hydrolysable phosphorus, and the fraction not hydrolysable by bone phosphatase under stated conditions. 3. The chemical nature of these fractions is discussed.

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14 Mai, H., *Z. Kinderheilk.*, 1928, **45**, 653.

15 Lawaczek, H., *Biochem. L.*, 1924, **145**, 351.

9234 P

Localization of Foreign Proteins and Dyes in Neoplastic Growth.

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The permeability of the stroma of malignant tumors of mice, both transplanted and spontaneous, has been investigated by the intravenous injection of a variety of foreign sera and dyes. The relative amount of the sera localized in the tumor was determined by the use of immunological methods and the dyes could be easily detected by direct examination under water.

After the intravenous injection of 0.25 cc. sera from the horse, rabbit, or chicken, precipitation tests on the extracts of the tumor, various organs, and the blood showed that, in the early period after injection (24 to 48 hours), a larger amount of the foreign sera was in the blood and tumor than in any of the organs.

After 3 to 5 days the sera from chicken and rabbit had disappeared from the blood and were found only or mostly in the tumor. Horse serum persisted in the blood for at least 10 days, but the tumor tissue always contained more of it than the blood.

The dyes which were tested are T. 1824, Pontamine Sky blue, Brilliant Vital red, Vital New red, Dianil blue, and others, diluted at 1:1,000. These were injected intravenously at the amount of 0.25 to 0.50 cc. and were all found to localize selectively in cancer tissue with comparatively little localization in organs such as the liver and lymph nodes, where they might be expected to accumulate. The localization in the tumor takes place rapidly, the dye being clearly detected in the growth within one half hour after the intravenous injection. The maximum accumulation is accomplished in 12 to 20 hours. A broad zone of skin around the tumor is also deeply stained. The first 2 of the above series seem to give the most clear-cut results. T. 1824, a dye allied to Trypan blue, was selected for more extensive investigation. During the first few days after the intravenous injection of 0.25 mg., this dye is found localized in the healthy part of the tumor, the necrotic tissue remaining unstained. Ten days or more later, in the rapidly growing, transplantable tumors, the dye has disappeared from the healthy part of the tumor and is found accumulated in the necrotic part. Pontamine Sky blue and Dianil blue behave like T. 1824 in this respect; but a variety of other dyes localize in both the healthy and necrotic parts, and still others were found to stain only the necrotic parts.

All mouse tumors so far studied, spontaneous or transplanted, primary or metastatic, are deeply stained after injection of T. 1824, and even pin-point metastases in the lung and liver may be readily detected by their blue color. The results are equally definite with the Brown-Pearce epithelioma of the rabbit and a sarcoma of the chicken. Five or 10 cc. of the 1:1,000 solution was injected in these cases.

Examination of the fresh, stained tumor with the naked eye or with a magnifying glass reveals that, although the whole tumor appears blue, the dye is mostly fixed in the stroma of the growth. Microscopic observations have not been extensively carried out as yet. However, the preliminary work so far done seems to indicate that T. 1824 behaves like Trypan blue, according to the careful studies of Ludford.[1] The cancer cell would be surrounded rather than penetrated by the dye.

The dye itself is without effect on the growth rate of the tumor. As it is known to combine readily with protein materials, an attempt was made to combine it with a toxic substance. Both rattlesnake venom and *Bacillus paratyphosus B.* toxin rapidly lose their toxicity in combination with the dye, but in this combination the dye still localizes in the tumors. In spite of the gross loss of toxicity, there appears to be an effect on the growth rate of the tumor following the intravenous injection of the dye in combination with a toxin.

9235 P

Active Immunity to Experimental Poliomyelitis by Intranasal Route in *Macacus rhesus.**

S. D. KRAMER, L. H. GROSSMAN AND G. C. PARKER.

From the Laboratories of the Infantile Paralysis Commission of the Long Island College of Medicine.

We wish to report protection against intranasal infection with subsequent development of immunity in monkeys that had received instillations of pituitrin S and adrephine,† followed by intranasal instillations of potent virus suspensions.

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1 Ludford, R. J., *Proc. Royal Society London*, B, 1929, **104**, 493.

* This work was supported by a grant from the Friedsam Foundation, and the President's Birthday Ball Commission for Infantile Paralysis Research.

† The pituitrin S and adrephine was in part supplied to us through the kindness of Parke, Davis & Company, Detroit, Michigan.

The treatment consisted of instilling ½ cc. of surgical pituitrin into each nostril, followed in 5 minutes by instillation of 1 cc. of adrephine (a preparation consisting of a mixture of adrenalin and ephedrine, with a small amount of chloretone).

One group of 16 animals received preliminary treatments with pituitrin S and adrephine twice a day for 5 and 7 days, followed by 3 daily instillations of 1 cc. of a 5% suspension of virus into each nostril (intranasal test). Of the 16 animals, 6 received the first of the instillations of virus 12 hours after the last treatment; 4 received virus 24 hours, 3 received virus 48 hours, and three, 96 hours after the last treatment. The animals were bled from 18 to 57 days after the intranasal test, and neutralization tests were done on their serums. A second intranasal test was performed on the surviving animals from 3 to 14 weeks later, and the animals surviving this test were inoculated intracerebrally with an infective dose of virus.

A second group of 10 animals received the instillations of pituitrin S and adrephine daily, followed in 4 or 6 hours by instillations of virus. Eight of the 10 animals received a second treatment with pituitrin S and adrephine, 4 hours after the virus. These daily treatments and instillations of virus were continued for 20 to 28 days. The surviving animals were bled and neutralization tests done on their serums. Then each animal received an instillation of one cc. of virus into each nostril on 3 successive days without pituitrin S and adrephine. The survivors of this intranasal test were then inoculated intracerebrally with a paralyzing dose of virus.

Eight of 10 of the 16 animals in the first group that received virus 12 and 24 hours after treatment survived the intranasal test and 2 succumbed to the experimental disease. Of the 6 animals that had received the virus 48 and 96 hours after treatment, one monkey, that had received virus 48 hours after treatment, survived, and 5 succumbed. Eight animals survived a second intranasal test. One animal died 25 days after the second intranasal test. Six of the 8 surviving animals neutralized the virus and 2 failed to neutralize. The 8 survivors were given the intracerebral test (.01 cc. of a 5% suspension of potent virus in 1 cc. of saline). Of the 8, two survived and 6 succumbed. One of those that succumbed came down after a prolonged incubation period (27 days), and a second animal developed a mild form of the disease and recovered.

Of the 10 animals that had received daily instillations of both pituitrin S, adrephine, and virus (second group), 6 survived daily treatments and exposure to virus and 4 succumbed to the disease within the incubation period of the virus. Neutralization tests were performed on the serums of these 6 animals. The serums of 3

neutralized the virus; one partially neutralized and 2 failed to neutralize. Five of these 6 animals survived the intracerebral test.

Of the 26 normal controls employed in the intranasal tests, 25 succumbed. Of the 11 normal controls employed in the neutralization tests, all succumbed, as did the 13 normal controls used in the intracerebral tests.

Histologic study of the mucous membranes of the animals treated with pituitrin S and adrephine shows an extensive infiltration of the superficial and deep layers of the mucosa with eosinophiles. Some scattered eosinophiles are present in the submucosa, otherwise the mucous membranes appear normal. Mucous membranes obtained from normal untreated animals do not show eosinophilic infiltrations.

It appears from the results of these experiments, that not only do the majority of the animals develop appreciable protection against intranasal instillations of potent virus, but an appreciable number of these animals, particularly those included in the second group that had received prolonged daily treatments with pituitrin S and adrephine, as well as virus, developed an active immunity as indicated by the neutralization and intracerebral tests. These results help to bring out even a greater similarity between the experimental and the human disease, and perhaps serve to support the epidemiologic concept of the mechanism involved in the production of a widespread immunity to the disease in the normal population.[1] We do not attempt to explain the significance of the eosinophilic infiltration (or "barrier") in the mucous membranes of the treated animals.

9236 P

Effect of Pancreatic Tissue Extract on Cholesterol of Blood in Cardiovascular Arteriosclerosis.* †

ANNA SAMUELSON. (Introduced by Israel S. Kleiner.)

From the Morrisania City Hospital, New York City.

Pancreatic tissue extracts have been studied since 1908 by various investigators. Since 1929, insulin-free extracts have been investigated and independently prepared by Frey, Gley, Kraut, Kisthinios, Vaquez, and Wolffe. Wolffe and his associates reported that their

1 Kramer, S. D., *J. A. M. A.*, 1932, **99**, 1048.

* Aided by a grant from the Lucius N. Littauer Foundation.

† I wish to thank Dr. Edward P. Flood, Director of Medicine, and Dr. William Aronson, Director of Laboratories, for their assistance and cooperation.

pancreatic extract possessed vaso-dilator properties, which antagonized the pressor action of epinephrine and caused a transitory lowering of blood pressure in the animal. The commercially prepared pancreatic tissue extracts‡ contain traces of histamine, choline, salts, foreign proteins, and even slight traces of insulin. It has been demonstrated by the latter investigators, that the epinephrine neutralizing property of this substance is probably not due to these contaminants.[1]

Work has been carried on for some time on the effect of pancreatic tissue extract in cardiovascular arteriosclerosis manifested by angina pectoris or precordial pain and intermittent claudication in peripheral vascular diseases. During the course of this investigation an alteration in the blood cholesterol and phospholipids was observed. Before treatment in these cases, the cholesterol content of the blood was found to be rather high, the average being 275 mg. per 100 cc. Following the administration of pancreatic extract, it was noted that along with some clinical improvement, there was a tendency for the blood cholesterol to drop.

The Kirk, Page and Van Slyke gasometric microdetermination method was used for estimating the plasma lipids.[2] The percentage of error for blood cholesterol was found to be less than 2%.

In this investigation, the presence of choline in pancreatic tissue extract No. 568, was demonstrated by the Kraut test. Blood sugar concentration was not altered after the administration of pancreatic tissue extract. Blood sugar determinations were made by the Folin Wu method simultaneously with the lipids, fasting, one hour and 24 hours after the injection of pancreatic tissue extract.

A control experiment was carried out on 18 ward cases chosen at random. In these, fasting blood cholesterol concentration was determined according to the Bloor colorimetric method before, and one hour after, the injection of (a) 5 cc. aqua distillata in 3 cases; (b) 5 cc. physiological normal saline in 5 cases; and (c) 5 cc. pancreatic tissue extract in 10 cases. In (a) there was an average rise of 4.9 mg. cholesterol per 100 cc. blood, one hour after injection; in (b) an average rise of 20.6 mg.; and in (c) an average decrease of 38.8 mg.

Results are summarized in Table I. One hundred and eighteen

‡ Tissue Extract No. 568 was prepared according to the method of Wolffe (*cf.* Hyman and Parsonnet, *The Failing Heart of Middle Age*, 1932, p. 442), by Sharp and Dohme, for which I wish to thank them.

1 Wolffe, J. B., *et al.*, reported before the 15th International Congress of Physiologists, Leningrad, August, 1935.

2 Kirk, E., Page, I. H., and Van Slyke, D. D., *J. Biol. Chem.*, 1934, **106**, 203.

TABLE I.

Effect of Tissue Extract on Total, Free and Esterified Cholesterol.
Columns marked (1)—represent Fasting concentrations.
" " (2)—one hour after injection of Pancreatic Tissue Extract.
" " (3)—24 hours " " " " " "
Esterified cholesterol may be calculated by subtracting Free Cholesterol from Total Cholesterol.

Subject Case No.	Date	cc. injected	Total Cholesterol			Free Cholesterol		
			1	2	3	1	2	3
1	10-20	10	270.0	195.1	214.8	65.8	90.9	74.8
2	10-6	10	196.4	230.0	254.2	91.2	68.8	71.1
3	8-20	5	306.5	213.7	—	115.5	110.6	—
	10-1	5	300.0	289.0	—	106.0	103.2	—
	10-29	5	279.5	260.0	270.0	145.0	138.5	121.5
	11-12	5	248.5	241.0	251.0	—	—	—
4	8-27	5	306.5	267.0	—	137.7	130.5	—
	10-1	5	243.0	210.0	—	163.5	120.9	—
	10-29	5	339.5	306.5	316.0	141.0	120.5	123.5
5	9-3	5	256.2	211.3	—	148.3	130.5	—
	10-1	5	335.0	318.0	—	136.0	159.0	—
6	10-22	5	373.5	341.0	328.5	163.0	148.5	146.5
	11-26	3	313.0	300.5	278.5	—	—	—

plasma cholesterol determinations were done on 23 patients after 46 injections. Forty-eight were done on fasting blood; 46 one hour after, and 24, 24 hours after injection of pancreatic tissue extract. In all but one case, a drop in the blood cholesterol was noted one hour after injection. Case 1 is a typical example. The exception (Case 2) showed a rise one hour after injection. There was a consistent fall in the blood cholesterol of 14 patients who had regularly received a 3 cc. injection 3 times a week for a period of time. In these, determinations were made at intervals of 3 to 5 weeks during the course of treatment. Case 3 is a typical example. Where the treatment was interrupted or dosage too small, *i. e.*, 1 or 2 cc. pancreatic tissue extract 3 times a week, there was an exacerbation of symptoms and an increase in plasma cholesterol. Such results were noted in 4 cases, demonstrated by case 4 (interrupted treatment) and case 5 (inadequate treatment). Of 24 determinations, on blood taken 24 hours after injection, 3 have shown a further fall below fasting and 21 tended to return almost to original level. Case 6 is a typical example of a continued fall 24 hours after injection.

The greatest drop in cholesterol in one hour was 97 mg. per 100 cc. blood or 31%. The mean decrease in cholesterol observed one hour after 5 cc. injection of tissue extract (37 injections) was 8.3%; the average deviation from the mean was 4.36%. The mean

decrease in cholesterol observed one hour after 10 cc. injection of tissue extract (9 injections) was 12.7%; the average deviation from the mean was 9.7%.

Summary. The results indicate that pancreatic tissue extract produces a lowering of the plasma cholesterol within one hour in cardiovascular arteriosclerosis. The cholesterol remains lowered, but with a tendency to return almost to former level in 24 hours. The effect is transitory, lasting about 24 hours, more or less. For sustained effect and progressive drop in plasma cholesterol, patients require doses at frequent intervals. When treatment is interrupted or dosage inadequate, the plasma cholesterol rises and there is an exacerbation of symptoms, precordial pain and intermittent claudication.

9237 P

Active Form of 2-4 Dinitrophenol in the Stimulation or Inhibition of Oxygen Consumption of Excised Rabbit Muscle.*

A. W. MARTIN AND J. FIELD, II.

From the Department of Physiology, Stanford University.

Field, Martin and Field[1, 2, 3] have shown that the stimulation or inhibition of yeast respiration by 2-4 dinitrophenol (DNP) and several related compounds depends upon the concentration of the undissociated form. Since this finding appears to have important physiological and pharmacological implications, we have undertaken an investigation of the action of DNP on excised rabbit striated muscle (diaphragm) to see whether the undissociated form is the active agent in stimulation of oxygen consumption of a mammalian tissue as well as of yeast.

Rabbits were killed by a blow on the back of the neck. The diaphragm was rapidly excised, and strips of proper thickness (*c. f.* Warburg[4]) were placed in Ringer's solution containing 0.2% glucose and buffered at the desired pH with M/150 phosphate. Res-

* Supported in part by a grant from the Rockefeller Fluid Research Fund of the Stanford University School of Medicine.

1 Field, J., II, Martin, A. W., and Field, S. M., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 56.

2 Field, J., II, Martin, A. W., and Field, S. M., *J. Cell. and Comp. Physiol.*, 1934, **4**, 405.

3 Field, J., II, Martin, A. W., and Field, S. M., *J. Pharm. and Exp. Ther.*, 1935, **53**, 314.

4 Warburg, O., *Uber den Stoffwechsel der Tumoren*, Berlin, 1926.

piration was measured by the Warburg manometric method[4] at 37° ± 0.02°C. in an atmosphere of oxygen. The respiration of each piece of tissue was followed for 30 minutes before addition of DNP from the vessel sidearms, to obtain a preliminary control period in each case. In addition to these individual controls in at least one case in every run (6 to 13 vessels), Ringer's-glucose was added from the sidearms instead of DNP to determine the rate of decrease of oxygen consumption with time under the conditions of these experiments over a longer period.

TABLE I.

Showing effects of graded concentrations of DNP, total and undissociated, on oxygen consumption of rabbit striated muscle (diaphragm) in Ringer's-glucose at pH 6.2, 7.2 and 8.0. DNP concentrations given in millimols. Plus indicates stimulation, minus inhibition. Optimum effects in italics.

Total DNP Concentration	% effect at pH			Undissociated DNP Concentration	% effect at pH		
	6.2	7.2	8.0		6.2	7.2	8.0
2.23 x 10–4	+38						
				2.34 x 10–7			+21
4.46 x 10–4	+34	+4					
				2.88 x 10–7		+4	
1.11 x 10–3	+120						
				4.68 x 10–7			+46
2.23 x 10–3	+162	+22	+21				
				1.44 x 10–6	+38	+22	
3.33 x 10–3	*+192*						
				2.33 x 10–6			+35
4.46 x 10–3	+97	+29	+46				
				2.88 x 10–6	+34	+29	
1.11 x 10–2	+123	+58					
				3.49 x 10–6			+60
2.23 x 10–2	+56	*+207*	+35				
				4.68 x 10–6			+94
3.33 x 10–2		+177	+60				
				7.18 x 10–6	+120	+58	
4.46 x 10–2	—19	+184	+94				
				1.17 x 10–5			*+252*
1.11 x 10–1		+200	*+252*				
				1.44 x 10–5	+162	*+207*	
2.23 x 10–1		+77	+63				
				2.15 x 10–5	*+192*	+177	
1.11		—26	—8				
				2.33 x 10–5			+63
2.23			—35				
				2.88 x 10–5	+97	+184	
				7.18 x 10–5	+123	+200	
				1.17 x 10–4			—8
				1.44 x 10–4	+56	+77	
				2.33 x 10–4			—35
				2.88 x 10–4	—19		
				7.18 x 10–4		—26	

Calculations of percentage stimulation (Table I) were based on the assumption that had no DNP been added the rate of decrease of oxygen consumption after the initial control period would have been the same as that observed in the longer control experiments. Controls showed this assumption valid within ± 5%. Accordingly, no change in oxygen consumption was considered significant unless it exceeded 10%.

If undissociated DNP is the active agent in the stimulation or inhibition of tissue respiration by this substance, one would expect to find the optimum total concentration to be a function of pH, while the optimum concentration in terms of the undissociated form should be quite constant. Furthermore, it should be possible to find a total DNP concentration which would stimulate respiration at one pH level and inhibit at another. It is shown in Table I that these expectations were realized. Such results are most directly explicable on the assumption that, over the pH range investigated, undissociated DNP is the active agent in the stimulation or inhibition of oxygen consumption of rabbit striated muscle (diaphragm).

9238

Metabolism of Anesthetized Rats.

M. KLEIBER AND F. J. SAUNDERS.*

From the College of Agriculture, University of California, Davis.

The experiments reported in this paper were undertaken chiefly in order to find out whether or not anesthesia could be used with advantage in metabolism studies as a means of reducing the variability of metabolic rates between different rats.

Female rats weighing from 65 to 163 gm. were anesthetized with a suspension of one per cent sodium amytal in 0.9% NaCl solution. The effect of intraperitoneal injection of the drug was tested in 15 rats. When 2 out of 3 rats injected intraperitoneally with 1.5 to 2 cc. of the suspension per 100 gm. of body weight died, the dosage was reduced, yet the mortality among the intraperitoneally injected rats remained high, so that only 6 results of this group could be used for comparison. The dosage of amytal for these intraperitoneally injected rats varied from 7.6 to 10.4 with an average of 9.2

* The authors are indebted to C. F. Winchester for the measurement of the respiratory exchange of the rats.

mg. per 100 gm. of body weight. The drug was injected subcutaneously into 11 female rats in doses of 5.8 to 9.8 with an average of 7.4 mg. per 100 gm. of body weight. Five litter mates served as controls for the intraperitoneally injected rats and 7 for the subcutaneously injected rats. The controls were so selected, that their body weights matched those of the experimental rats.

Prior to the injection of the anesthetic the experimental rats as well as the controls were fasted for 24 hours. The respiratory exchange of each rat was determined individually in a closed respiration apparatus of the Regnault-Reiset type. Seven such apparatuses are combined in a thermostatic cabinet and are operated simultaneously. The respiration trials were started approximately one-half hour after injection of the experimental rats and lasted over a period of 6 hours during which time the oxygen consumption was recorded every half hour. The CO_2 production was determined at the end of the trial for the entire period.

In order to eliminate the influence of body size on the results, the amount of oxygen consumed per half hour was divided by the 3/4 power of body weight. The results thus obtained with the individual rats of each of the 4 groups (2 groups of injected rats and 2 corresponding groups of controls) were averaged for each of the half-hour periods of the respiration experiment. The standard deviation for one result expressed in per cent of the respective mean is the coefficient of variability for the metabolism of different rats of one group within one period. The average of these coefficients of variability for all half-hour periods was $\pm 12.4 \pm 1.2\%$ for the intraperitoneally injected and $\pm 12.5 \pm 1.0\%$ for the corresponding control rats. The average coefficient of variability for the subcutaneously injected rats was $9.0 \pm 0.5\%$, that for the corresponding controls amounted to $13.3 \pm 1.1\%$.

Anesthesia decreased the metabolism of the rats considerably. During the first half-hour period of the respiration experiment, the rate of O_2 consumption of the intraperitoneally injected rats was only 68%, that of the subcutaneously injected rats only 80% of the rate of oxygen consumption of the respective controls. This depressing influence of the drug gradually disappeared in time and during the last 3 periods of the respiration experiment, *i. e.*, later than 5 hours after the injection the metabolic rates of the injected rats were practically equal to those of the controls. In order to eliminate individual differences in the metabolic level the results for each rat in the various periods of the experiment were expressed in per cent of the average results of the last 3 periods for the same rat. The relative figures thus obtained for the injected rats in each

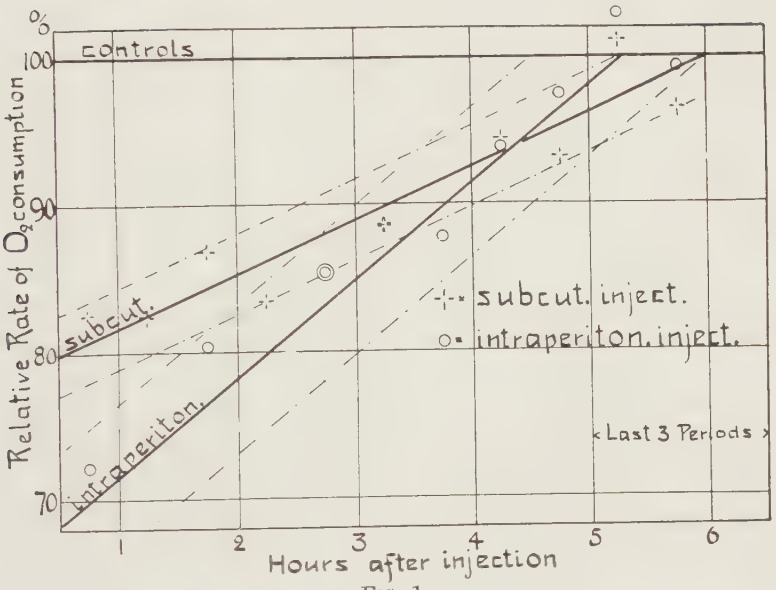

FIG. 1.

Relative rate of oxygen consumption of anesthetized rats in per cent of the corresponding rate of control rats.

period were in turn expressed in per cent of the average for the control rats in the same period. This calculation was carried out in order to eliminate possible systematic influences of time as such on the metabolic rates of the rats. The results obtained are plotted in Fig. 1. The crosses indicate the relative oxygen consumption of the subcutaneously injected rats; the circles represent the corresponding data for the intraperitoneally injected rats. The double cross and the double circle indicate data obtained during two periods together. The heavy straight lines in the figure illustrate the result of linear interpolation of the data by the method of least squares which led to the following equations:

$$M_i = 68.2 + 6.66\ (t - 0.5)$$
$$M_s = 79.9 + 3.64\ (t - 0.5)$$

where M_i = oxygen consumption of the intraperitoneally injected rats in per cent of the corresponding values for the controls

M_s = corresponding results for the subcutaneously injected rats

t = time in hours after the injection

The difference in the regression coefficients ($6.66 \pm 0.72 - 3.64 \pm 0.40$) which determine the slope of the two lines in the figure is statistically highly significant. Its random probability is below one per cent. The dash point lines on the figure indicate the boundaries of the standard deviation of the lines.

The results indicate that amytal injected intraperitoneally had a more pronounced effect on metabolism but lost this effect faster than did the drug injected under the skin.

The total effect of the drug on metabolism may be measured as the product of the decrease in metabolic rate and the time. This total effect is indicated on the figure as the area of the triangle formed by the ordinate at the start of the experiment, the inclined straight line and the horizontal line which represents the metabolic rate of the controls. This total effect contains an element of uncertainty because the respiration trials started approximately half an hour after the injection of the drug. The total effect on the subcutaneously injected rats amounted to 73% of the effect on the intraperitoneally injected rats. The dosage of amytal per unit of body weight in the subcutaneously injected rats amounted to 80% of the dosage applied intraperitoneally. This difference may partly explain the difference in the total effect. The differences in dosage within each group, however, did not systematically affect the rate of metabolism per unit of the 3/4 power of body weight, measured during the entire period of 6 hours, which averaged to 93.8 ± 4.3 Cals. daily for the intraperitoneally injected, and to 91.8 ± 2.0 Cals. for the subcutaneously injected rats.

Conclusions. Intraperitoneal injection of amytal did not decrease the variability in the rate of oxygen consumption between different rats. Subcutaneous injection of the drug decreased this variability so that average results of a given standard deviation of the mean could be obtained with approximately half as many rats under anesthesia as would be required with non-treated rats. It is questionable whether or not this decrease in variability justifies the application of anesthesia in respiration trials since this application may introduce influences on metabolism tending to make the results less conclusive in many cases even though the variability may be decreased. The application of the anesthetic also makes the metabolic rate dependent on the time after injection so that a result on an anesthetized rat is not conclusive unless the time after the injection and its influence on the rate of metabolism are defined.

9239 P

Tobacco Sensitization in Rats.

Joseph Harkavy. (Introduced by Louis Gross.)

From the Laboratories of Mount Sinai Hospital, New York City.

Friedlander, Silbert and Laskey[1] reported the occurrence of gangrene of the toes in male albino rats injected daily with denicotinized tobacco by the intraäbdominal route. In order to elucidate the nature of these lesions it was deemed important to determine whether the tissues of the animals thus injected had been sensitized to tobacco. We therefore repeated their experiments and, having confirmed their observations, selected those animals in whom we had succeeded in reproducing the lesions described by Friedlander, *et al.*, for this investigation.

The present report deals with results obtained by testing intestinal strips of 6 injected animals and 5 controls.

Of the 6 treated animals one was injected with tobacco only; 4 with horse serum, eggwhite, and tobacco; and one with ragweed only. Injections were given daily until the toe-lesions appeared. This usually occurred in 6 to 10 weeks. After the appearance of gangrene, injections were stopped for about 10 days and the animal sacrificed for our experiments. The extracts used in injecting the animals represented a mixture of tobaccos prepared from the cured leaves of Burley, Maryland, Virginia and Xanthis tobaccos, denicotinized and extracted according to a technic previously described.[2] This extract contained 0.085 mg. N per cc. The ragweed-pollen extract contained 0.42 mg. N per cc.; the eggwhite 1.2 mg. N per cc. The horse serum was furnished by the Board of Health and used in undiluted form. The amount of the total material injected never exceeded one cc.

The animal to be studied was killed by a blow on the head and about one inch of the duodenum or intestine excised. This was suspended in a bath containing 75 cc. of oxygenated Tyrode's solution. When the movements of the intestinal strip had stabilized, the extract with which the animal had been injected was introduced into the bath, and its effect on the gut recorded.

The amount of tobacco-extract used was determined in each instance by preliminary standardization on the suspended intestinal

1 Friedlander, M., Silbert, S., and Laskey, N., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 156.

2 Harkavy, J., and Romanoff, A., *J. Allergy*, 1934, **6**, 56.

strip obtained from a normal rat. This enabled us to learn the effect of our tobacco extract on the excised intestine of normal rats and also to determine the optimal amount that we could use in studying the reaction of the tissue obtained from the injected animal.

No phenol was used in preparation of the test-extract, because we found that it had a paralyzing effect on the intestinal strip.

The preëstablished quantity of the tobacco-solution (usually between 0.2 and 0.5 cc.) was introduced into the bath containing the intestinal strip of the injected animal. In each one of the 5 tobacco-injected rats it caused a prompt contraction. After the preparation was washed, subsequent addition of the same quantity of tobacco-extract was without effect. This indicated desensitization of the gut. (Illustration.)

None of our 5 non-injected animals reacted to tobacco-extracts, even in doses of 1.6 cc. (Illustration.)

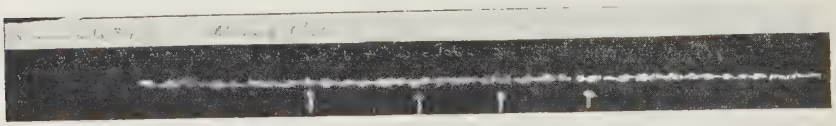

FIG. 1.

Negative response of intestinal strip of normal rat to repeated introduction of 0.2 cc. of tobacco extract. A total of .8 cc. of extract present in bath.

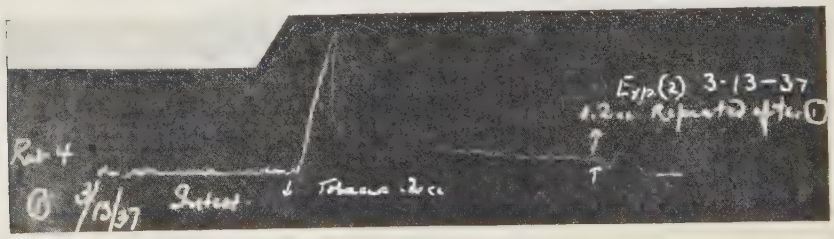

FIG. 2.

Exp. 1. Positive anaphylactic reaction of gut from rat 4, injected with denicotinized tobacco, following the introduction of .2 cc. of tobacco extract into bath.

Exp. 2. The same intestinal strip following the reaction in Exp. 1 giving a negative response to .2 cc. of tobacco extract indicating that the gut had been desensitized.

No evidence of sensitization to ragweed, horse serum, or eggwhite was obtained in animals 1, 2, 4, and 5, which had been injected with ragweed, horse serum and eggwhite in addition to tobacco. Animal 6, one of 3 injected with ragweed only, without having developed any lesions, when tested with tobacco and ragweed gave negative reactions to both. (Table I.)

These results indicate that 5 animals injected daily with tobacco

TABLE I.
Reactions of Intestinal Strip of Normal Rats and Rats Injected with Tobacco, Horse Serum, Eggwhite, and Ragweed.

Rat No.	Injected daily with	cc.	Toe lesions	Intestinal strip tested with	Results
1	a. Horse serum	0.5		0.1 cc. horse serum 1:100	Negative
	b. Tobacco	0.5	Positive	0.2 cc. tobacco*	Positive
2	a. Ragweed-pollen	0.5		0.3 cc. ragweed-pollen 0.42 mg. N per cc.	Negative
	b. Tobacco	0.5	"	0.2 cc. tobacco*	Positive
3	a. Tobacco	1.	"	0.5 cc. tobacco*	"
4	a. Horse serum	0.33		0.3 cc. horse serum 1:100	Negative
	b. Eggwhite	0.33	"	0.3 cc. eggwhite 0.1 mg. N per cc.	"
	c. Tobacco	0.33		0.2 cc. tobacco*	Positive
5	a. Horse serum	0.33	"	0.3 cc. horse serum 1:100	Negative
	b. Eggwhite	0.33		0.3 cc. eggwhite 0.1 mg. N per cc.	"
	c. Tobacco	0.33		0.2 cc. tobacco*	Positive
6	Ragweed-pollen	1.		0.3 cc. ragweed-pollen 0.42 mg. N per cc.	Negative
			Negative	0.4 cc. tobacco*	"
7-11	Non-injected		"	Tobacco	"

*Tobacco extract contained .085 mg. N per cc.

alone, or tobacco plus ragweed, horse serum or eggwhite, who had developed gangrenous lesions of the toes within 6 to 10 weeks, gave definite evidence of sensitization to tobacco but no sensitization to any of the other extracts used.

Our inability to demonstrate sensitization to the other injected substances such as ragweed, horse serum or eggwhite is not without precedent. With the exception of Arthus in 1903 and J. T. Parker and F. Parker in 1924, numerous investigators, including Longcope who used horse serum, have failed to sensitize rats.

Experiments involving passive transfer, anaphylactic shock and cross-desensitization are being carried out in order to elucidate further the nature of the reaction described.*

* I wish to express my appreciation to George Brooks for his technical assistance.

9240 P

The Electroencephalogram During Infancy and Childhood.

J. Roy Smith. (Introduced by Rustin McIntosh.)

From the Normal Child Development Study of the Department of Diseases of Children, Columbia University, and the Babies' Hospital, New York.

The study of the electroencephalogram in infancy and childhood has thus far received little attention from investigators. Of the existing data those of Lindsley[1] (*c.f.* also Berger,[2] Loomis, Harvey and Hobart,[3] Davis and Davis,[4] and Kreezer[5]) showing the nature of the frequency variation of the so-called "alpha waves" with age are the most complete. In the present study observations have been made which are in general corroboration of Lindsley's findings except for one minor difference. In addition data have been obtained which indicate the presence within a few days after birth of previously unreported rhythmic waves apparently different from the alpha waves and emanating from the motor region.

Observations were made upon 58 normal children ranging in age from one day to 17 years. The group included 13 infants and young children upon whom serial observations were made at intervals of 7 to 30 days over a period of several months. In order to record the alpha waves which in the adult are usually observed over the occipital lobes, standard electrode positions over this region (both electrodes on the midline, the anterior one just posterior to the lambda and the inter-electrode distance 3½ to 4½ cm.) were employed. Recording technique including amplifiers and ink-writing undulators was standard. Tracings were taken with the child lying at rest and in semi-darkness within a shielded chamber.

Under these conditions the potentials appearing during the first 2½ months are predominantly random and "base line" in appearance. During the second month irregular sequences of waves of alpha amplitude (50μv approximately) may be observed but it is not until the tenth or twelfth week that short series of rhythmic waves varying between 3 and 4 oscillations per second begin to appear. During this early period (tenth to fourteenth week) repeated measurements on the same infant show that these rhythmic waves are

1 Lindsley, D. B., *Science*, 1936, **84**, 354.

2 Berger, H., *Arch. f. Psychiat.*, 1932-33, **98**, 231.

3 Loomis, A. L., Harvey, E. H., and Hobart, G., *J. Exp. Psychol.*, 1936, **29**, 249.

4 Davis, H., and Davis, P. *Arch. Neurol. Psychiat.*, 1936, **36**, 1214.

5 Kreezer, G., *Arch. Neurol. Psychiat.*, 1936, **36**, 1206.

quite variable, being well-defined one week and hardly noticeable the next. By the end of the fourth month, however, they are usually well-established and can be reproduced consistently. Thereafter they gradually increase in frequency and reach the adult level (approximately 9 oscillations per second) at about 8 years. This gradual increase in the frequency of the alpha waves over the period from the third month to the eighth year is in general agreement with Lindsley's observations. Our data, however, fail to show any acceleration beyond the adult average from the tenth to the twelfth years such as he has reported.

While no rhythmic activity is observed over the occipital region before the third or fourth month with the above described technique, simultaneous tracings obtained from electrode positions over other underlying cortical areas do show periodic waves long before this time. These waves appear only when the infant is asleep; they may be present within a few days after birth and may be of various rates and amplitudes. One example of this early rhythmic activity is given in Fig. 1, which shows tracings taken simultaneously from 3 separate areas on the scalp in a sleeping infant 12 days old. As shown in the figure, the upper tracing is taken from the occipital region. The electrodes for the middle tracings are over the motor

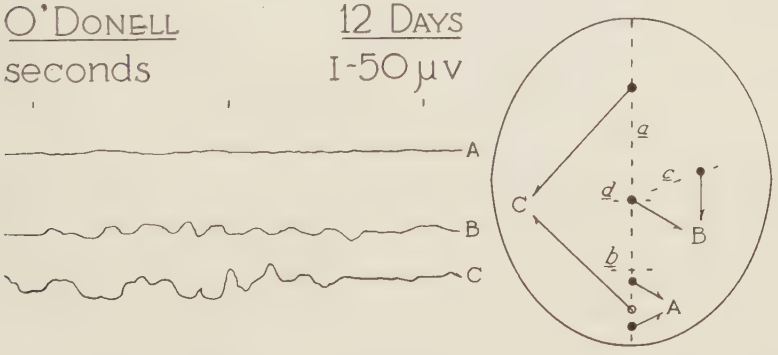

FIG. 1.

Brain potentials from sleeping infant 12 days old. The paired electrode positions are shown in approximate relation to underlying cortical structures in the schematic figure at the right. Upper tracing, leads A: occipital lobes over midline *a* posterior to parieto-occipital sulcus *b;* middle tracing, leads B: motor region approximately over central sulcus *c* with medial lead over superior Rolandic point *d;* lower tracing leads C: occipital to frontal over midline. Time interval is one second. All amplifiers equated at the gain indicated.

Note the presence of well-defined periodic waves at about 5 per second over the motor region and the absence of any rhythmic waves over the occipital area.

region, one being on the so-called "superior Rolandic point" and the other 4 cm. away at an angle of 70° with the midline, anterior to

this point and thus approximately over the central sulcus. The electrodes for the lower tracing include between them practically the entire cortex along the midline, one being over the mid-occipital region and the other just anterior to the anterior fontanelle. The 3 amplifiers are equated in gain. It is to be noted that while there is no semblance of any rhythmic activity over the occipital region, well-defined periodic waves at approximately 5 per second are present over the motor region. These latter waves as shown by the lower tracing are not quite so well-defined when the leads include the whole cortex between them. Repeated observations have shown that the essential conditions for the appearance of these waves during the first few weeks after birth are that one or both electrodes should be over the motor region or that the latter area should be included between the electrodes, and that the child should be asleep. Their probable origin in the motor region is thus indicated.

Waves at 4 to 5 per second similar to those already described have been observed as early as the first post-natal day. They do not appear to be the forerunner of the familiar 10 per second alpha waves but rather of large slow rhythmic waves at 4 to 5 per second which have regularly been observed in older children during states of drowsiness and light sleep and which also appear to originate in the motor region. It should be noted that Kreezer[5] has also reported large slow waves at 4 to 5 per second appearing over the motor area in mongoloid defectives with mental ages below 5 years.

Rhythmic waves at 8 per second, as well as faster, smaller oscillations varying from 12 to 15 per second have also been observed within a few days after birth. These latter waves also have their counterparts in comparable frequencies which have been observed repeatedly in older children during sleep. The faster, smaller waves particularly have been found by the writer to appear typically over the motor region and only during deep sleep. They probably correspond to the 14 per second waves reported by Loomis, Harvey and Hobart[3] as appearing during sleep in adults.

9241

An Improved Thunberg Technique for Bacterial Oxidations.

Frank H. Johnson.* (Introduced by E. Newton Harvey.)

From the Laboratory of General Physiology, Princeton University.

Although more accurate methods of measuring redox potentials in connection with bacterial oxidations are now available,[1, 2] the Thunberg technique,[3] which has been extended by Quastel[4, 5] and others, will no doubt continue to find wide application. It is especially useful where a large number of determinations are to be made, because of its greater simplicity and less time-consuming nature than the electrometric methods. At the same time, however, there are important sources of error. The purpose of this paper is to describe an improved method which either totally eliminates or greatly reduces the errors associated with the Thunberg technique as it has generally been used.

In a recent critical study, Yudkin[6, 7] points out 6 factors which may interact to alter the reduction time of methylene blue by bacteria, when the suspension is evacuated and placed in a water bath. These are: (1) the temperature lag effect, (2) presence of residual oxygen, (3) gradual poisoning of the enzyme by methylene blue, (4) affinity of the enzyme for methylene blue, (5) presence of hydrogen donators in the suspension, and (6) the products of the action of the bacteria on the substrate which can act as hydrogen donators. The evacuation has nearly always been carried out for less than 2 minutes, with a water aspirator. Since Harvey[8] has shown that the rate of reduction of methylene blue by dehydrogenases in milk is proportional to the reduction in oxygen tension, the question naturally arises concerning the efficacy of one or 2 minutes' evacuation. This point was tested by luminous bacteria, which constitute a very good indicator for the presence of oxygen,[9] for their light dims at a partial pressure of about 2 mm. of mercury[10] and remains visible at 10^{-5} atmospheres.[11]

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* Eli Lilly and Company Research Fellow.

1 Korr, I. M., *J. Cell. Comp. Physiol.*, 1935, **6**, 181.

2 Yudkin, J., *Biochem. J.*, 1935, **29**, 1130.

3 Thunberg, T., *Skand. Arch. f. Physiol.*, 1920, **40**, 1.

4 Quastel, J. H., and Whetham, M. D., *Biochem. J.*, 1924, **18**, 519.

5 Quastel, J. H., *Erg. Enz. Fors.*, 1932, **1**, 209.

6 Yudkin, J., *Biochem. J.*, 1933, **27**, 1849.

7 Yudkin, J., *Biochem. J.*, 1934, **28**, 1454.

8 Harvey, E. Newton, *J. Gen. Physiol.*, 1919, **1**, 415.

9 Hill, S. E., *Science*, 1928, **67**, 374.

10 Shoup, C. S., *J. Gen. Physiol.*, 1929, **13**, 27.

11 Harvey, E. N., and Morrison, T. F., *J. Gen. Physiol.*, 1923, **6**, 13.

To 3 standard-type Thunberg tubes, each containing 1 cc. of buffer solution in the side arm, were added 9 cc., 6 cc., and 3 cc., respectively, of a very dilute suspension of luminous bacteria in phosphate buffer, pH = 7.30. They were connected with a Hyvac pump and exhausted continuously. A mercury manometer in the system indicated that a vacuum approximately equal to the vapor pressure of water was quickly reached. But the time for the complete removal of the oxygen, as judged by the complete extinction of luminescence, was 19 minutes for the first tube, 26 for the second, and 36 for the third. A 10 cc. portion of the same suspension in a test tube indicated a dimming time without evacuation (due only to the using up of oxygen by the respiration of the organisms) of approximately 36 minutes. Furthermore, when the buffer was

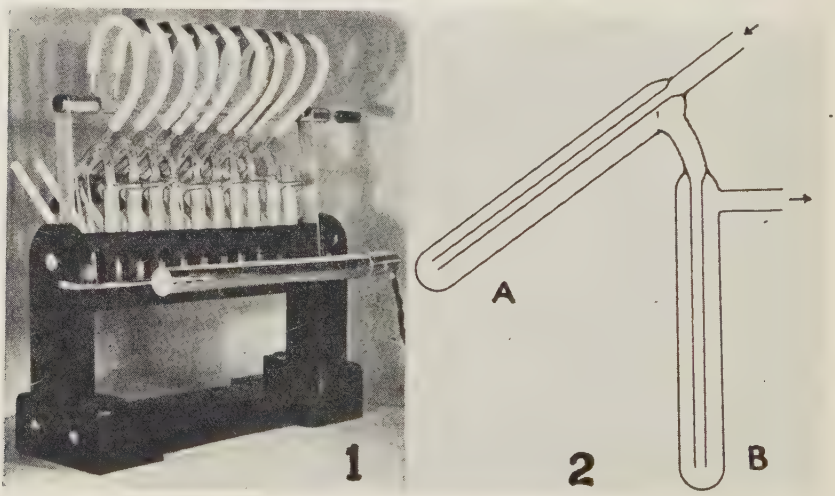

FIG. 1.

Apparatus for simultaneous deaeration of a series of the tubes illustrated in Fig. 2. The manifold is attached to the rack so that the whole unit can be rotated on its long axis near its base, thus mixing the substrate plus methylene blue solutions of all tubes with the bacteria at the same moment. When restored to the upright position, decolorization of the dye is observed, from the opposite side, against the long electric light globe (held within the split brass tube) as a source of illumination. For *perfect* deaeration lead tubing should be used entirely in conducting the gas to the vessels, but thick rubber tubing as illustrated has been found quite effective and easy to manipulate. The manifold can be slipped out of the brass holders to remove or replace vessels. The latter are loaded while tilted to the rear. The dowls prevent the rear arms of the vessels from swinging sideways during tilting.

FIG. 2.

Vessel for separate deaeration of substrate plus methylene blue solutions (in arm A) and the bacterial suspension (in arm B). The solutions and the suspension, respectively, are washed down by the final addition of a buffer solution. After use, the vessels are readily cleaned by cleaning solution followed by running water.

emptied from the side arms to the suspension in the evacuated tubes, a bright flash of light occurred, showing the presence of dissolved oxygen. This light faded rapidly, but went completely out only after several minutes. It was not restored by shaking the tubes, which were now practically rid of all oxygen.

These facts clearly indicate that even with prolonged evacuation, the final removal of the oxygen depends on the respiration of the organisms, and indeed, under ordinary conditions, the latter is probably by far the more important factor. In order to insure complete anaerobiosis, without depending on respiration to use up the oxygen, deaeration by a stream of pure hydrogen or pure nitrogen is the most effective means, and by using for this purpose a type of tube introduced by Harvey[12] and suggested by him for use in this connection, the bacterial suspension can be deaerated separately from methylene blue and substrate solutions. (Fig. 2.) Separate deaeration followed by anaerobic mixing eliminates possible errors in reduction rate arising from the following causes: (1) the aerobic metabolism of the substrate by the bacteria, before the complete removal of oxygen, (2) the influence of different substrates in stimulating respiration to different degrees, and hence to different rates of oxygen removal, (3) substrates that cause little or no increase in oxygen consumption, but which act as hydrogen donators anaerobically,[13] (4) substrates that actually inhibit oxygen consumption, but which may reduce methylene blue anaerobically (alpha methylglucoside; see below), (5) substances which inhibit aerobic metabolism of substrates, but which are without effect on anaerobic dehydrogenases,[14] and (6) substances which may inhibit anaerobic dehydrogenase activity without appreciably affecting aerobic respiration.[14] In addition, separate deaeration, by shortening the reduction time, minimizes the toxic action of the dye, and since it can be carried out at a constant temperature completely avoids the temperature lag effect.

Taking advantage of these principles, the apparatus pictured in Fig. 1 was constructed. With this arrangement a series of tubes can be completely deaerated with hydrogen purified over hot platinized asbestos, or nitrogen purified over hot copper, within a very few minutes. The substrate and dye solutions in the rear arms can then be added to the bacterial suspension in the front arms at exactly the same time. The data in Table I indicate how this method can avoid some fundamental errors.

12 Harvey, E. Newton, *Biol. Bull.*, 1926, **51**, 89.

13 Cook, R. P., *Biochem. J.*, 1930, **24**, 1538.

14 Keilin, D., *Proc. Roy. Soc. Lond.* (B), 1930, **106**, 418.

TABLE I.

Time for 90% reduction of methylene blue (1:50,000) by a phosphate buffer suspension of luminous bacteria (*Achromobacter Fischeri*), with and without added substrate.

Suspension	Substrate	Reduction time		
		Deaerated by evacuation	Deaerated by hydrogen	Deaerated by nitrogen
Unwashed	—	27′	7′	7′ 15″
Washed 4 times	—	>3 days	>6 hr.	>6 hr.
″ ″ ″	M/10 glucose	27′	8′	8′
″ ″ ″	M/10 alpha methyl glucoside	>3 days*	29′	29′

*Barely perceptible decolorization in 6 hrs, that amounted to approximately 30% reduction over a period of 3 days.

It is a matter of considerable importance that the usual technique of evacuation gave only a vague evidence for the dehydrogenation of alpha methylglucoside. This substance has been found to inhibit aerobic respiration of washed cells[15] and would thus lengthen the time for removal of oxygen. Yet the new method shows that it is readily dehydrogenated. In addition to use with bacterial suspensions, this method should work equally well with any tissue or tissue preparation which can be delivered with a pipette.

9242

Effect of Testosterone on Somatic Growth.

C. S. McEUEN, H. SELYE AND J. B. COLLIP.

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It has been shown by Steinach and Holzknecht[1] that ovarian hormones inhibit growth. They found that implantation of ovaries into male guinea pigs or rats decreased their growth rate in every case if the transplant took. Later, these findings were confirmed by Bugbee and Simond,[2] and Spencer, *et al.*,[3, 4] using oestrin preparations. Spencer, *et al.*,[5] came to the conclusion that the growth in-

15 Johnson, Frank H., *J. Cell. Comp. Physiol.*, 1936, **8**, 439.

1 Steinach, E., and Holzknecht, G., *Archiv. f. Entwcklngsmechanik d. Organ.*, 1916, **42**, 490.

2 Bugbee, E. P., and Simond, A. E., *Endocrinol.*, 1926, **10**, 360.

3 Spencer, J., Gustavson, R. G., and D'Amour, F. E., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 500.

4 Spencer, J., D'Amour, F. E., and Gustavson, R. G., *Am. J. Anat.*, 1932, **50**, 129.

5 Spencer, J., D'Amour, F. E., and Gustavson, R. G., *Endocrinol.*, 1932, **16**, 647.

hibition is due to a depression of pituitary function by oestrin. Retardation of growth in the rat under the influence of oestrin administration has also been reported by Korenchevsky and Dennison[6] and Halpern and D'Amour.[7] While the growth hormone production of the pituitary is thus interfered with, the actual size of the hypophysis increases so much that tumor formation may occur (Zondek,[8] Cramer and Horning,[9] McEuen, *et al.*[10] The inhibition of pituitary function is also evidenced by the atrophy of the gonad which ensues under the influence of oestrin administration. This has been shown by Herrmann and Stein[11] who, with oestrogenic placental and corpus luteum extracts, obtained testicular atrophy in the rat and other mammals. In the female rat, gonad atrophy was observed following the injection of oestrogenic ovarian extracts or follicular fluid by Terada.[12] Moore and Price,[13] who made similar observations with purified ovarian hormone preparations, advanced the theory that injections of gonadal hormones inhibits the production of gonad-stimulating hormone by the hypophysis. The fact that oestrin may cause gonad atrophy was later confirmed repeatedly (Wade and Doisy,[14] Ihrke and D'Amour,[15] Leonard, *et al.*,[16] Spencer, *et al.*[5]).

In connection with these experiments it seemed of interest to establish whether testosterone would have similar effects. In order to obtain evidence concerning this question we treated rats chronically with large doses of testosterone.* The first series consisted of 12 normal and 9 castrate males, and 12 normal and 11 castrate females. It was divided into two groups as shown in Table I. The first group received daily subcutaneous injections of 200γ of testosterone in corn oil, beginning at the 25th day of life. Their controls, the second group, received a similar dose of cholesterol in oil or oil alone. In the normal males treated with testosterone the testes and scrota were obviously much smaller than in those not

6 Korenchevsky, V., and Dennison, M., *Biochem. J.*, 1934, **28**, 1486.

7 Halpern, S. R., and D'Amour, F. E., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 108.

8 Zondek, B., *Lancet*, Jan. 4, 1936, p. 10.

9 Cramer, W., and Horning, E. S., *Lancet*, May 9, 1936, p. 1056.

10 McEuen, C. S., Selye, H., and Collip, J. B., *Lancet*, Apr. 4, 1936, p. 775.

11 Herrmann, E., and Stein, M., *Zentralbl. f. Gynäkol.*, 1920, **44**, 1449.

12 Terada, M., *Japan. med. World*, 1927, **7**, 233.

13 Moore, C. R., and Price, D., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 1.

14 Wade, N. J., and Doisy, E. A., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 714.

15 Ihrke, E. A., and D'Amour, F. E., *Am. J. Physiol.*, 1931, **96**, 2.

16 Leonard, S. L., Meyer, R. K., and Hisaw, F. L., *Endocrinol.*, 1931, **28**, 714.

* The authors are indebted to Dr. Erwin Schwenk of the Schering Corporation for the testosterone used in these experiments.

TABLE I.

	Duration of treatment days	Body weight increase in gm.					
		Testosterone			Controls (oil)		
		No. of rats	Extreme individual variations	Aver.	No. of rats	Extreme individual variations	Aver.
Normal males	432	6	334-374	353	6	273-394	334
Castrate* "		5	301-437	358	4	330-475	380
					Controls (cholesterol)		
Normal females	272	6	164-260	220	6	139-201	169
Castrate "		5	184-260	230	6	173-253	204

*Injections were omitted during 54 days owing to lack of supply of Testosterone.

TABLE II.

Males	No. of rats	Body weight increase in gm.		Aver. endocrine weights				
		Extreme individual variations	Aver.	Testes	Sem. Vesicles and Prostate	Epidid.	Adrenals	Pituitary
Testosterone	5	60-100	75.4	1.324	1.557	.415	.023	.007
Cholesterol	5	59- 89	77.6	2.133	.365	.327	.027	.006
Untreated	5	59- 97	80.2	2.429	.830	.419	.024	.007
Females				Ovaries				
Testosterone	5	53- 77	63.8	.016			.022	.007
Cholesterol	5	34- 53	48.6	.031			.034	.006
Untreated	5	34- 51	44.2	.037			.032	.006

treated with this hormone. The normal females treated with testosterone went into permanent vaginal dioestrus within the first 2 months of treatment, while the cycles in the cholesterol treated controls remained normal. The organ weights of this group are not recorded in the table as the animals have been kept alive for further observation.

In a second series, consisting of 15 normal males and 15 normal females, 5 animals of each sex received the high dose of 2 mg. of testosterone in corn oil daily by subcutaneous injection. Five other males and 5 females received the same dose of cholesterol in oil, while the remaining 5 of each sex were left untreated. The animals were 36 to 38 days of age at the initiation of treatment and they were sacrificed on the 23rd day of the experiment. Their somatic weights and the weights of their endocrines are summarized in Table II. In all testosterone treated animals the testes and ovaries showed atrophy and the females became dioestric within the first 7 days of testosterone treatment, while no such change was observed in any of the control groups. Histologically the mammary glands of all the testosterone treated animals were well developed and showed some secretion, while no development or secretion was observed in untreated or cholesterol treated females and only slight development was seen in the untreated males. The fact that the mammary gland of the normal rat (not that of the castrate) shows some development and that testosterone will stimulate the development of the mammary gland, both in the male and in the female castrate, has been described in previous communications.[17, 18] As is seen in Tables I and II there is no sign of somatic growth inhibition in either group under the influence of testosterone; indeed, there appears to be a suggestion of growth stimulation, at least in the normal female. The hypophyses likewise failed to show any stimulation comparable with that obtained by us[19] with oestrin under similar conditions.

Summary. No somatic growth inhibition was observed in the rat even when treated with very large doses of testosterone, although these same doses proved sufficient to inhibit gonad development in both sexes. It has been found that doses of testosterone which suffice to inhibit the growth of the gonad in both sexes and to cause

17 Selye, H., McEuen, C. S., and Collip, J. B., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 201.

18 McEuen, C. S., Selye, H., and Collip, J. B., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 56.

19 Selye, H., Collip, J. B., and Thomson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1377.

permanent vaginal dioestrus in the female do not cause hypertrophy of the hypophysis in either sex. In these respects, the effect of testosterone on somatic and hypophyseal growth differs from that of oestrone.

9243

Action of Arasaponins A and B.

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The Chinese drug San-ch'i has been botanically identified as *Gynura pinnatifida*,[1, 2] although it has been named *Aralia bipinnatifida*[3] by Y. H. Chao. The plant is a short herb grown in southwestern China. The root is the part that has been advocated in Chinese medicine chiefly as an astringent and hemostatic. In a previous note,[3] one of us (T. Q. C.) reported the isolation of 2 saponins from San-ch'i, arasaponin A and arasaponin B. The former melts at 195-210°C., has a specific rotation $[\alpha]_D$ +23°, and conforms to the empirical formula $C_{30}H_{52}O_{10}$; while arasaponin B melts at 190-200°C., is also dextro-rotatory ($[\alpha]_D$ +8°), and has an empirical formula $C_{23}H_{38}O_{10}$. Both substances are moderately soluble in water, foam forming upon agitation.

Hemolysis experiments, 14 in all, were carried out according to the method described by Ponder[4] with both saponins at 37°C. It was found that arasaponin A laked a guinea pig's red cells in the concentration of 1:4000 within 2 hours 43 minutes. Solutions of 1:2500, 1:2000, 1:1000, 1:750, 1:500, and 1:250 were more readily effective, but it still required 1 hour 14 minutes. Weaker concentrations, such as 1:5000, 1:8000, and 1:10,000, had no hemolytic action at the end of 8 hours. Arasaponin A also hemolyzed dogs' and monkeys' blood, but the latent period was very long. For example, a 1:250 solution laked a dog's red cells in 4 hours 34

[1] *Botanica Nomenclature*, Commercial Press, Shanghai, 1917, p. 23.

[2] Chen, C. J., *Encyclopedia of Chinese Materia Medica*, The World Press, Shanghai, 1924, **1**, 38.

[3] Chou, T. Q., and Chu, J. H., *Proc. Chinese Physiol. Soc.*, Tsingtao Meeting, 1936, p. 12.

[4] Ponder, E., *The Mammalian Red Cell and the Properties of Hæmolytic Systems*, G. Borntraeger, Berlin, 1934, p. 139.

minutes, and the same concentration caused complete lysis in a monkey's erythrocytes in 17 hours 44 minutes. No hemolytic action was observed with arasaponin A upon rabbits', sheep's, or pigeons' red blood cells.

Arasaponin B hemolyzed the red corpuscles of guinea pigs, dogs, and rabbits, although it required a long latent period even with concentrated solutions. For example, a concentration of 1:250 laked a guinea pig's erythrocytes in 3 hours, a dog's in 4 hours 36 minutes, and a rabbit's in 5 hours 16 minutes. No hemolysis took place with monkeys', sheep's, or pigeons' blood at the end of 24 hours, a 1:250 solution of arasaponin B being employed.

When injected intravenously into an etherized cat, neither arasaponin altered the level of blood pressure, the heart rate, or the respiratory rate, in doses varying from 1 to 20 mg. Similarly, no effect was noted on the motility of the isolated guinea pig's uterus, or the isolated rabbit's intestines, in concentrations of 1:50,000 and 1:25,000.

TABLE I.
Toxicity of Arasaponins A and B in Albino Mice by Intravenous Injection.

Arasaponin	Dose, mg. per kg.	No. of mice injected	No. of mice died	Minimal lethal dose, mg. per kg.
A	300	2	0	460
	350	2	0	
	370	2	0	
	400	2	0	
	420	5	1	
	440	5	1	
	460	5	4	
B	200	2	0	300
	220	2	0	
	240	2	0	
	260	4	0	
	280	5	2	
	300	5	3	
	320	2	2	

As shown in Table I, the minimal lethal dose of arasaponin A is 460 mg. per kg. in white mice by intravenous injection, and that of arasaponin B 300 mg. per kg. A 2% solution was used in each case. Death, as a rule, was prompt, although a few animals lingered for 20 to 30 minutes, or longer. A group of 6 guinea pigs survived doses of arasaponin A or B varying from 100 to 300 mg. per kg., administered by the saphenous vein. Erection of hair was the only sign observed. Gold fish immersed in arasaponins A and B for 24 hours were not affected in concentrations of 1:500, 1:1000, and

1:10,000, except in one instance a fish finally died in a 1:1000 solution of arasaponin A.

Summary. The pharmacologic action of arasaponins A and B, isolated from the Chinese drug San-ch'i, has been investigated. Arasaponin A possesses a hemolytic action on the erythrocytes of guinea pigs, dogs, and monkeys. It has a minimal lethal dose of 460 mg. per kg. in mice by intravenous injection. Arasaponin B has a hemolytic action upon the red blood cells of guinea pigs, dogs, and rabbits. Its minimal lethal dose in mice is 300 mg. per kg. when injected by the tail vein.

9244

Prolonged Splanchnic Stimulation.*

FREDERICK A. FENDER. (Introduced by Frederick L. Reichert.)

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An attempt was made to determine the effect of prolonged bilateral electrical stimulation of the splanchnic pathways of a dog upon the level of systolic blood pressure.

Blood pressure records were made by connecting a van Leersum carotid flap to a "Tycos" recording sphygmomanometer by means of especially constructed rubber bags and rigid tubing. The method has been checked against optical manometry and gives results consistently about 10% high; more than 2000 determinations in 8 dogs convinced us of its efficiency.

Stimulation was provided by a method involving the use of surgically implanted secondary units excited by a primary field in which the animal is free to move.[1] A frequency of 60 cycles was used at 6-8 volts.

Following construction of a carotid flap the subject, a robust 12-kilo male terrier, was trained over a period of several weeks to lie quietly during blood pressure determinations. In the following 3 weeks 89 records were made to serve as a base line. The average systolic pressure during this time was 143 mm. Hg. (all figures refer to records obtained by our method). Then and subsequently, except when the conditions of an intercurrent experiment dictated other-

* Work done as Fellow of the Rockefeller Foundation. Financed by grants from the Committee on Scientific Research of the American Medical Association.

[1] Fender, Frederick A., *Am. J. Physiol.*, 1936, **116**, 47.

wise the systolic pressure for any one time was considered to be the average of 4 records with a "spread" of less than 15 mm. Hg. The average deviation of the daily levels, obtained in this way, from the period average, was 2.5 mm. Hg.

November 4, 1935, a secondary unit with bipolar electrodes loosely encircling the splanchnic nerve just above the diaphragm, was implanted on each side. On the fourth postoperative day a second control period of 22 days began. Eighty-four determinations in this period varied between 146 and 166 with an average of 156 mm. Hg. The slight rise was thought to be due to mechanical irritation of the splanchnics, as there was no indication of discomfort.

Preliminary tests showed that stimulation (animal in cage) resulted in agitation and in a sharp rise in systolic level. The week of December 2-9, short periods of stimulation were used. Records made before the animal was put in the cage for stimulation averaged 141 mm. Hg. for the week. Records made at least 2 hours after one-second periods of stimulation averaged 163 mm. Hg.

Stimulation was then increased so as to keep the subject's systolic blood pressure elevated throughout the day. For 5½ months the subject's systolic level was kept elevated 8 hours a day, 6 days a week. This level varied between 160 and 200 mm. Hg., with an approximate average of 175. In addition to daily routine determinations, frequent checks carried out during the "stimulation day", and the conduct of the animal under stimulation, satisfied us that a real elevation was maintained.

Stimulation was always followed by agitation as well as by an increase in blood pressure. However, 2 experiments under completely effective pentobarbital anesthesia in which sharp elevations of from 25 to 30 mm. Hg. were obtained with stimulation, showed that the effect was not wholly due to emotional factors.

In spite of the continued elevations during the day, the animal's systolic level did not remain elevated without stimulation. The last 16 morning determinations, made May 11 to 16 before the animal entered the cage, ranged from 120 to 137 mm. Hg. with an average of 128.

May 23, 1936, it was noticed that the animal could avoid stimulation partially by selecting a certain position in the cage, and it was suspected that one of the coils had ceased to function. This proved to be the case at autopsy, May 29, when it was found that a terminal on the right coil had parted. The sympathetic trunks and splanchnic nerves showed no evidence of trauma, and a careful examination of the organs by Dr. Klaus Rosencrans, especially inter-

ested in the pathology of hypertension, showed no significant departure from the normal.

Throughout the experiment the animal's blood urea level was followed at weekly intervals. There was no change. Dr. David Rytand made repeated examinations of the urine without discovering significant changes.

Conclusion. The systolic blood pressure of a dog was maintained at an elevated level 8 hours a day, 6 days a week, for 5½ months without producing any lasting effect on the resting level.

9245 P

Synthesis of Octopine (Pectenine).

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The compound isolated by Moore and Wilson[1] from Pecten muscle and called pectenine by them is probably identical with octopine previously isolated by Morizawa.[2] The arguments in favor of this statement will be given in a forthcoming publication.

Octopine was characterized by Moore and Wilson and, on evidence which was not quite complete, they concluded that the compound is probably arginine, the α-amino group of which is attached to the α-carbon atom of propionic acid. In order to confirm this conclusion a synthesis has been carried out as follows[3]: d-arginine methyl ester dihydrochloride was treated with the ethyl ester of d-l, α-bromopropionic acid in absolute ethyl alcohol containing a little zinc dust and potassium iodide. Two equivalents of sodium ethylate were added at once and another equivalent added during the early part of the period of boiling which was continued for 24 hours. The esters were hydrolyzed with acid and the material was precipitated by silver and baryta. After decomposing the precipitate, the small amount of arginine was removed with flavianic acid and a picrate was obtained from the filtrate after extraction of the flavianic acid.

The recrystallized picrate melted with decomposition at 219°.

1 Moore, E., and Wilson, D. W., *J. Biol. Chem.*, 1934, **105**, lxiii; *Am. J. Med. Sci.*, 1935, **190**, 143; *J. Biol. Chem.*, 1936, **114**, lxxi.

2 Morizawa, K., *Acta Scholæ Med. Kioto*, 1927, **9**, 285.

3 Ruzicka, L., and Fornasir, V., *Helv. Chim. Acta*, 1920, **3**, 806.

Octopine picrate melted at 224° and a mixed melting point was 219° (all uncorr.). The new picrate when analyzed by the Jorpes modification of the Sakaguchi method gave 97% of the expected color when octopine was used as a standard. The color given by the new picrate with the Sakaguchi reagents was identical with the purple color given by octopine. Picric acid analysis yielded the following results: Calculated for $C_9H_{18}N_4O_4.C_6H_3N_3O_7$ 48.20%, found 48.31%. The compound showed no free amino nitrogen with the Van Slyke method. The specific rotation, after removal of the picric acid, was +10° while the specific rotation of natural octopine is +20°. The free compound was crystallized from water by adding alcohol. The synthetic material melted at 257-60°; octopine melted at 266-68° and a mixture melted at 264-67° (uncorrected. All melted with gas evolution). The pH of dilute water solutions of both the synthetic and natural compounds was about 6.4. These results suggest that we have prepared partially inactive octopine. We plan to treat d-arginine with optically active bromopropionic acid.

9246

Quantitative Assay of Insulin Effect.

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In the post-absorptive state the constancy of the blood sugar level is an expression of a well-maintained balance between glycogenolysis and the withdrawal of sugar from the blood stream. The fall of the blood sugar level subsequent to an injection of insulin is regarded as the result of an inhibition of the glycogenolytic process, the withdrawal of blood sugar going on unaltered.[1, 2, 3] The degree to which the blood sugar is lowered is not in direct proportion to the insulin dosage.[4] One may increase the insulin dosage considerably in the lower and higher ranges with little or no increase in the degree of blood sugar depression. The effect of the larger dose expresses

1 Issekutz, B. von, *Biochem. Z.*, 1927, **147**, 264; **148**, 283.

2 Sahyum, N., and Luck, J. M., *J. Biol. Chem.*, 1929, **85**, 1.

3 Cori, G. T., Cori, C. F., and Buchwald, K. W., *Am. J. Physiol.*, 1930, **93**, 273.

4 Scott, E. L., and Dotti, Louis B., *Arch. Int. Med.*, 1932, **50**, 511.

itself in a maintenance of the maximum depression for a longer time period and in a slowing of the rate of return to the initial level. It follows that the effect of injected insulin lasts as long as the blood sugar is below the post-absorptive level. The quantitative determination of insulin action must, therefore, involve the measurement of both the intensity of blood sugar depression and its duration.

The times chosen by MacLeod and his coworkers[5] for determining blood sugar as a measure of insulin effect are suitable on the basis of the above postulate when relatively large doses are employed (0.5 units per kilo or over). Himsworth[6] has sought to measure the sensitivity of man to insulin by measuring the area enclosed by the depression curve below the resting level for the first 15 minutes. Scott and Dotti (loc. cit.) measured insulin response in terms of blood sugar depression at what they regard as optimum interval: 30 minutes after administration. They found that the proportionality between the blood sugar change and insulin dosage over a range of dosages from 1/16 to ½ unit per kilo followed a logarithmic rather than a direct relationship. Neither of the 2 latter insulin blood sugar relationships is an adequate measure of insulin action in the sense that it is an inhibitor of the glycogenolytic process in the liver. Scott and Dotti's method applicable in the study of large groups does not serve well for small numbers.

Insulin action in its entirety seems best expressed by the area confined by the blood sugar curve and a line parallel to the abscissa which intersects the curve at the initial post-absorptive level. No direct proportionality exists between insulin dosage and such areas even under strictly uniform experimental conditions in the same animal on a uniform diet. For example, in 4 dogs given 0.3 and 0.15 units per kilo at a 2-day interval the average ratio of the areas was 1.57, the individual variations being considerable. In 12 normal dogs given 0.5 and 0.25 units per kilo the areas of response at a similar interval showed a ratio of 3.73, the individual variations being less than in the previous group. It was found consistently that in the lower dosage ranges doubling the dosage causes considerably less increase in insulin action than in the higher dosage ranges. The inference is made that in the former a relatively larger quantity of insulin is required for the initiation of the insulin effect than is left to carry it on. Variable factors in the glycogenolytic system preclude proportionality in the complete insulin effect. The effect of 2 insulin samples or of 2 different dosages of the same

[5] MacLeod, J. J. R., *Carbohydrate Metabolism and Insulin*, 1936, Longmans, Green and Company, Ltd., London.

[6] Himsworth, H. P., *Clin. Science*, 1935, **2**, 67.

insulin preparation is considered identical when they produce blood sugar curves of similar form and magnitude. Under this condition all the variables, known and unknown, are so integrated as to represent the equivalent of a single factor, the insulin dosage.

Such a method of insulin assay has been roughly applied by us to measure the change in insulin response in dogs at 4 weeks and at 12 months after hypophysectomy. Under uniform conditions it was found that intravenous insulin 0.25 units per kilo produced blood sugar curves similar to those obtained by the use of 0.5 units per kilo before operation (Fig. 1). Twelve months after operation these animals were approximately 4 times as sensitive as when normal.

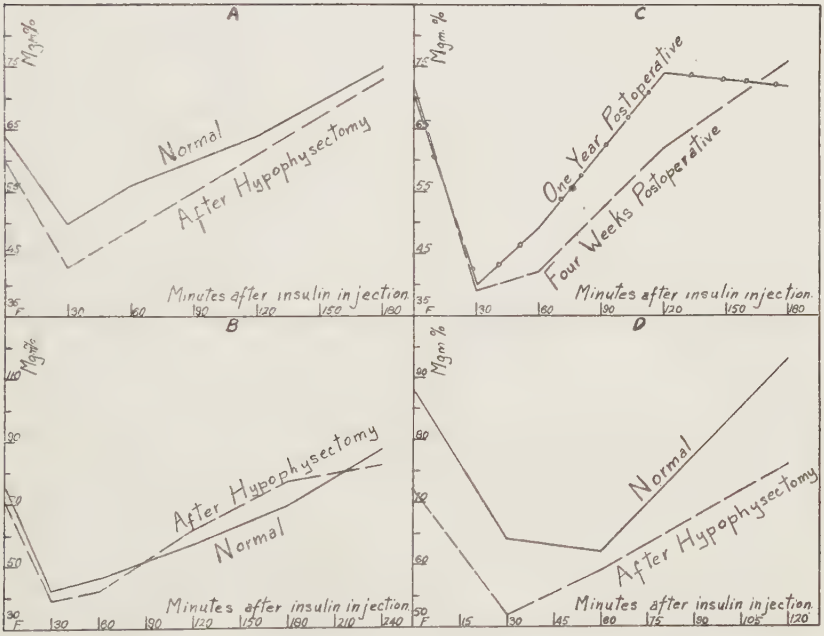

FIG. 1.

A. Blood sugar curves illustrating insulin effect in a dog before and 4 weeks after hypophysectomy. Preoperative dosage was 0.5, postoperative 0.25 units per kilo. The curves are approximately comparable.—Normal curve unbroken curve, after hypophysectomy broken.

B. Similar curves on a second dog 4 weeks after hypophysectomy. Insulin dosages as in A.

C. Blood sugar curves from same animal as for B. Dosages were 0.25 units 4 weeks after operation and 0.125 units per kilo one year after hypophysectomy.

D. Blood sugar curves on same animal as for C preoperatively and one year after operation. Preoperative dosage 0.25 units, postoperative 0.06 units per kilo. Note that C and D both indicate a postoperative insulin effect approximately four times that found for this dog preoperatively.

9247

Physicochemical Changes of the Blood in Experimental Thrombopenic Purpura.*

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A broader understanding of the mechanism of hemostasis requires, among other things, a systematic investigation of the physical and chemical constants of the blood during disturbances of that function. The present report summarizes observations on the venous pressure and viscosity of the blood, the colloid osmotic pressure and proteins of the plasma at various stages of thrombopenic purpura produced in dogs experimentally with antiplatelet serum.

The type of dog, their diet and living conditions, the method for preparation and standardization of the serum, counting platelets, measuring clot retraction, the mean bleeding time and petechial reaction of the skin have been described elsewhere.[1, 2, 3] In the present group the bleeding time was done on at least 3 locations in the skin of the abdomen and thorax and discontinued if it exceeded 900 seconds. Venous pressure was measured by the direct method with a 22 gauge needle connected by a 2-way stop-cock to a manometer filled with salt solution. Pressures were taken, with a minimum of trauma, in the arm and leg vein each time, with the animals lying horizontally and the extremity held at the approximate level of the heart; at least 10 seconds were allowed for stabilization of the pressure. Viscosity of the blood and plasma were measured in a Hess viscosimeter at 20°C. (±0.5°), critical negative pressures of 50 mm. Hg. for plasma and 100 mm. Hg. for blood being used to move the columns of liquid. The average of at least 5 readings was taken each time. Colloid osmotic pressure of the plasma was measured in duplicate, in Wells' micro osmometer,[4] with collodion membranes having permeability numbers between 15×10^{-8} and 30×10^{-8}, and adhering in all details to Wells' technique. Plasma proteins were analyzed in duplicate by the macro Kjeldahl method. Globulin was precipitated at 38°C. with 22.5% sodium sulphate.[5]

* Aided by a grant from the J. Ewing Mears Research Fund. Miss Katherine Pierpoint rendered technical assistance.

1 Tocantins, L. M., *Arch. Path.*, 1936, **21**, 69.

2 Tocantins, L. M., *Ann. Int. Med.*, 1936, **9**, 838.

3 Tocantins, L. M., *Am. J. Clin. Path.*, 1936, **6**, 160.

4 Wells, H. S., *Am. J. Physiol.*, 1932, **101**, 409.

5 Howe, P. E., *J. Biol. Chem.*, 1921, **49**, 109.

Fibrinogen was analyzed as fibrin by the method of Cullen and Van Slyke.[6] Nitrogen values were converted into protein values by multiplying by the factor 6.25. Non-protein N was determined in the plasma filtrate after precipitation of the protein with 10% trichloracetic acid. Each group of determinations was done in a single sample of blood collected without stasis from the jugular vein of the fasting animal into a syringe containing a small amount of a 25% solution of potassium oxalate. This introduces a dilution of a little less than 2%. Studies were carried out before, during and after the thrombopenia induced by a slow intravenous injection of a moderate dose of antiplatelet serum (0.1 cc. per kilo body weight). Eight experiments were performed on 5 animals. Three of the animals received 2 injections, not more than 4 days

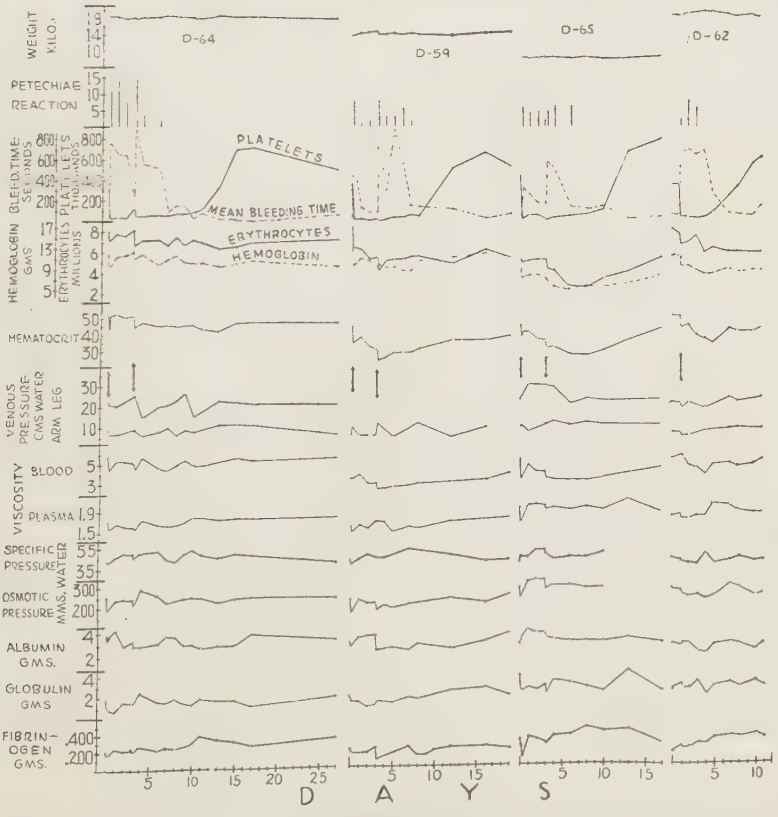

FIG. 1.

Effect of the injection of antiplatelet serum (indicated by arrows) on 4 dogs. The first determination after each injection was performed 2 hours after the serum was administered.

6 Cullen, G. E., and Van Slyke, D. D., *J. Biol. Chem.*, 1920, **41**, 587.

apart; the remaining 2 received a single injection each. A figure was considered significant when it differed from the mean normal for that determination by more than 2½ times the standard deviation. Each correlation coefficient was calculated from groups of 76 to 107 pairs of variables and was considered significant if it exceeded 6 times its probable error. Results in 4 dogs are illustrated in Fig. 1.

There was little difference between the findings in animals that were given 2 injections and those that received a single one, although in the former the period of thrombopenia was twice as long as in the latter. Changes in the blood 2 hours after an injection were less marked after the second injection. With exception of the blood viscosity and non-protein nitrogen, most changes during and after the phase of purpura were unimportant and could be attributed chiefly to variations in concentration of the blood as a result of circulatory disturbances induced by injection of the antiserum. For example, 2 hours after an injection nearly all measurements showed a decrease of approximately equal magnitude (averaging about 20%). In the absence of information on fluctuations of the blood and plasma volume at various periods of the experiment, it is not possible to state how much these changes were due to dilution or concentration of blood. The experiments were intended primarily, however, to find out whether any characteristic changes in the physicochemical properties of the blood occurred when the tendency to prolonged bleeding existed; therefore, the prevailing values, whether they were the result of blood dilution or concentration, should answer this point. Since nearly all values decreased 2 hours after an injection, the regularly occurring increase in non-protein N, although small (+19.8%), must have some significance. Because this N was not fractionated, it is not possible to state in what fraction the increase occurred. In dogs that lost a moderate amount of blood the decrease in total cell volume led to a significant diminution in blood viscosity. The average percent decrease in percent cell volume and in blood viscosity in the animals given 2 injections was about the same (−34.2% and −33.7% respectively) and occurred about 4 days after the second injection. In those that received a single injection the decreases were similar in magnitude (−29.9% for the percent cell volume and −33.6% for the blood viscosity) and appeared about the 3d day after the injection. There was a high direct correlation between the percent volume of cells in the blood and blood viscosity (+0.803±0.027). Although the correlation between the blood viscosity and mean bleeding time was not significant (−0.319±0.069), the decrease in blood viscosity during an at-

tack of purpura might influence bleeding, not by affecting its duration, but by allowing a greater volume output of blood per unit time from the wound. An increase in mean output of blood per second from wounds is often found in clinical and experimental thrombopenias.[3]

There were several significant correlations between the physicochemical variables themselves, which will be taken up elsewhere. Besides those already stated, there were few significant zero or first order correlations between the mean bleeding time, number of platelets, hematocrit, clot retraction, petechiæ reaction and each of the physicochemical variables. One of the highest correlations found was between the amount of fibrinogen and mean bleeding time (-0.413 ± 0.059), thus reinforcing clinical knowledge of the occasional association, causal or otherwise, between fibrinopenia and prolonged bleeding from wounds. Within the range of values observed in this study there was no significant correlation between the degree of clot retraction and the fibrinogen content of the blood ($+0.036 \pm 0.73$) thus supporting the fact that qualitative changes in the fibrin play a more important part in determining that property of the clot, other things being equal, than quantitative changes.[7] There was a moderately high, direct correlation between the number of blood platelets and the total protein content of the plasma ($+0.403 \pm 0.063$). Changes in globulin were perhaps largely responsible for this significant correlation, since disintegration of platelets is said to increase the globulin content of the medium[8] and, of the proteins studied, the globulin fraction showed the greatest correlation with the number of platelets ($+0.304 \pm 0.064$). A decrease in globulin is observed in clinical and experimental thrombopenia,[9] an increase following a phase of thrombocytosis.[10]

The physicochemical forces studied do not apparently play a dominant part in the mechanism of disturbed hemostasis. Investigation of these forces was carried out because it was felt that the number of blood platelets alone did not adequately explain the disturbance of this mechanism in thrombopenic purpura.[2] Subsequent experiments have indicated that any correlation between platelets and bleeding must be established from counts on arterial blood[11] and that a volume increase in platelets may, at times, compensate partially for a deficiency in numbers.

7 LeSourd, L., et Pagniez, P., *J. de Physiol. et Path. Gener.*, 1913, **9**, 812.

8 Comhaire, S., Roskam, J., and Vivario, R., *Com. Rend. Soc. Biol.*, 1934, **117**, 72.

9 Jurgens, R., *Deut. Arch. klin. Med.*, 1931, **171**, 378.

10 Frey, H. C., *Deut. Arch. klin. Med.*, 1928, **162**, 1.

11 Tocantins, L. M., *Proc. Physiol. Soc. Phila., Am. J. Med. Sci.*, 1936, **192**, 150.

Summary. The blood of dogs with thrombopenic purpura induced by antiplatelet serum shows a moderate decrease in blood viscosity (directly correlated with a decrease in total cell volume) and a transient increase in non-protein nitrogen. The venous pressure, plasma viscosity, total and specific colloid osmotic pressure and plasma proteins do not undergo significant changes.

9248 P

Hyperalimentation in Normal Animals Produced by Protamine Insulin.

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Beginning some years ago there were clinical reports[1–5] that undernourished, non-diabetic patients gained weight under the influence of insulin. The latter is regarded as stimulating the appetite, leading to a higher caloric intake of food. It is now used for this purpose by many although good proof that it is efficacious is still lacking. The reason for this is because of the many factors involved in such clinical observations. Experiments on animals have been disappointing, such as the negative results recorded for rabbits.[6] In experiments on normal rats carried out some seven years ago with ordinary insulin we were unable to influence either the food intake or body weight. In an attempt to duplicate experimentally with protamine insulin the occurrence of fatty livers, which has been attributed to chronic hypoglycemia in patients,[7] we were surprised by the marked influence on alimentation. A typical experiment is presented in Fig. 1. Each group of rats was composed of 3 adult males of about the same weight. They were on a diet supplied *ad lib.* and containing casein 25, starch 40, butter fat 15, lard 10, brewers yeast 5 and standard salt mixture 5. Protamine zinc insulin* was given subcutaneously in doses of 8 units (0.2 cc.) per

1 Bauer, R., and Nyiri, W., *Med. Klinik*, 1925, **21**, 1454.

2 Boekheler, T., *Munch. Med. Woch.*, 1926, **73**, 1921.

3 Haemmerli, A., *Schweiz. Med. Woch.*, 1926, **56**, 1095.

4 Bauer, R., *Klin. Woch.*, 1928, **7**, 1743.

5 Fonseca, F., *Arch. f. Verdauungs Krankheit.*, 1928, **42**, 362.

6 Long, M. L., and Bischoff, F., *J. Nutrition*, 1930, **2**, 245.

7 Judd, E. S., Kepler, E. J., and Rynearson, E. H., *Am. J. Surg.*, 1934, **24**, 345.

* We are indebted to Eli Lilly & Company of Indianapolis for the supply of Protamine zinc insulin used in this investigation.

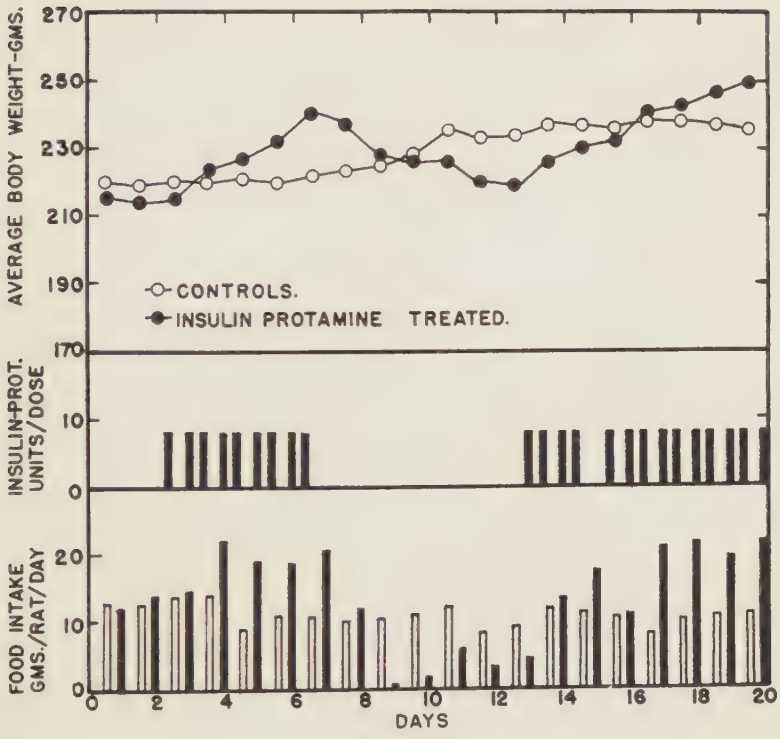

FIG. 1.

rat, twice a day as a rule. The increase in food intake is remarkable and there is a corresponding increase in body weight. When insulin injections are stopped the compensatory decrease in food intake is interesting. A food box was accidentally left out of the cage of one insulin-treated group for only 4 hours and all of the rats died in hypoglycemia before it was returned. Other details will be brought out in a later report. Our results suggest that whatever good results ordinary insulin may have in human undernutrition, the protamine compound will prove much better.

9249

Effective Stimulation of Crop-Sacs by Prolactin in Hypophysectomized and in Adrenalectomized Pigeons.

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Four years ago 2 of us[1] reported the stimulation of the crop-sacs of one completely (and of another incompletely) hypophysectomized pigeon with prolactin. Subsequently this result was confirmed by other tests which were not published. Recently Gomez and Turner[2] reported failure to obtain such stimulation, even with the sensitive local crop-sac method, on 16 completely hypophysectomized pigeons. Following acquaintance with their results our own series of unreported tests was increased sufficiently to provide a quantitative measure of the effect of hypophysectomy on the crop-sac response. These and other data relating to this response after hypophysectomy or adrenalectomy are presented here.

Hypophysectomy was done by the parapharyngeal route which permits the removal of the anterior pituitary with but little loss of posterior lobe tissue in most cases. Completeness of removal of anterior pituitary tissue (or of other extirpated tissue) was investigated with serial sections in all cases. Intramuscular injection only was employed. The chief criterion of crop-sac stimulation was its increased weight; but crop-milk formation was observed in most of the much prolonged tests as well as in slightly more than half the 4-day tests, and histological proof of active cell division (including colchicine test) was observed in the few cases thus studied at the end of the usual 4-day term of injection. Common pigeons of various races (and ages) were used. The "control" crop-sac weights listed in Table I are average values of unstimulated crop-sac areas obtained for groups (4 to 14) of unoperated birds of the same race.

The data of Table I make it fairly evident that when less than 5% of anterior pituitary tissue is present the crop-sac response (increase in weight) is hardly different from that in complete hypophysectomy; and our own fuller information indicates that the factor of *race*—together with the marked difference in body weights—is

[1] Riddle, O., Bates, R. W., and Dykshorn, S. W., *Am. J. Physiol.*, 1933, **105**, 191.

[2] Gomez, E. T., and Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 59.

TABLE I.

Growth Response in Crop-Sacs of Hypophysectomized (and Other Operated) Adult Pigeons to 4 Daily Injections of Prolactin.

No. of bird	Age when killed, mo.	Time since operation, days	Body wt., gm.	Prolactin daily, units	Weight of crop-sacs	
					Control, mg.	Found, mg.
		Hypophysectomy complete.				
1	16.3	177	530	30	1265	4800
2	26.0	191	428	30	1032	4700*
3	20.0	8	361	30	890	2755
4	16.2	8	298	30	800	2830
5	32.4	8	547	30	1265	4145
6	7.8	14	436	30	950	2865
7	8.8	14	326	30	726	2855
		Less than 1% pituitary present.				
8	17.1	8	516	30	1265	5475
9	9.5	8	426	30	925	5215
10	7.7	14	449	30	1050	2200
11	19.9	111	454	30	1025	6070
		Less than 5% pituitary present.				
12	28.8	170	264	30	726	5300†
13	18.1	287	374	30	1012	3915
14	27.5	162	572	5	1350	1665
15	20.6	116	280	5	726	895
		Adrenalectomized only.				
16	18.0	8	517	30	1300	7570
17	2.8	6	588	30	1300	5380‡
		Thyroidectomized only.				
18	33.0	180	279	15	630	4060
19	29.0	30	510	50	1025	17620§

*This bird also completely castrated 58 days before.

†Injected for 7 days (giving greater response than 4 days).

‡A trace of adrenal present on right side.

§Fragment (6.8 mg.) of one thyroid present; dosed with prolactin, thyrotropic and F.S.H. for 9 days.

chiefly responsible for the wide variation in the values obtained in birds Nos. 1 to 13. When we calculate[1] the crop-sac response of 12 of these birds (No. 12 omitted because injected for 7 days), each of which was injected with a total of 120 prolactin units, it is found that the weight of their crop-sacs correspond to only 63 units. Moreover, these 63 units must be divided by 4, since these crop-sac weights were obtained in *adults*—whose response averages about 4 times greater than that of the immature birds utilized in all original prolactin assays. Thus the average crop-sac weight obtained in these adult hypophysectomized pigeons is that expected to result from only 16 instead of 120 prolactin units; in other words, this response in the crop-sac of the hypophysectomized pigeon is only about one-eighth that of the normal mature pigeon. Injections were begun in this series of birds at periods ranging from 4 to 283 days after hypophysectomy.

It is clear that the crop-sacs of hypophysectomized pigeons are

effectively stimulated by prolactin; and equally clear that very much more prolactin is required to give a particular amount of proliferation than in the intact pigeon. Some impairment of crop-gland structure by this operation is scarcely a matter of surprise; it is still to be shown that there is any organ of the body which remains unaffected by complete removal of the anterior pituitary. We suppose that the quantity of prolactin injected (intramuscularly or intradermally) by Gomez and Turner was too small to give them a definite response in their pituitaryless birds.

Table I also lists data indicating that the crop-sacs of an adult adrenalectomized pigeon respond to prolactin when dosage was begun 4 days after this operation. For a completely thyroidectomized pigeon a like result was obtained from dosage beginning 176 days after thyroid removal.

TABLE II.

Growth Response in Crop-sacs of Very Young Completely Hypophysectomized Pigeons Dosed During 2 to 38 Days with Various Quantities of Prolactin.

No. of bird	Age (after hatching)		Body wt., gm.	Dosage		Wt. of crop-sacs found, mg.
	When operated, days	When killed, days		Prolactin daily, units	Duration, days	
20	12	29	230	25	18	2155
21	10	22	152	25	11	1440*
22	13	35	183	25	20	1415*
23	14	23	126	25	2½	970
24	12	28	170	25	4	710
25	8	21	116	6	12	885*
26	10	27	191	2	17	2705*
27	8	33	147	6	22	1280*
	Whole A.P. extract injected (prolactin content known).					
28	17	30	170	25	13	2435
29	12	21	152	25	9	2225
30	8	18	187	12	5	2780*
31	16	58	215	12	38	2150*
32	8	31	298	10	23	8510*

*More or less secretion present.

The data of Table II demonstrate that after removal of the pituitary from very young pigeons their crop-sacs respond to prolactin by growth and secretion. In all of the 13 tests—including the small crop-sacs (and short-term dosage) of Nos. 23 and 24—effective stimulation was obtained. In these tests dosage was started 0 to 12 days after operation and continued from 2 to 38 days. The daily dosage varied between 2 and 25 units. These variables of time, dosage, age and size provide data of some additional interest though we cannot supply control crop-sac weights (relatively larger in young than in adults) for these young squabs. In somewhat older,

and in adult, pigeons usual dosages of prolactin are continued during about 4 to 6 days before the secretion of crop-milk begins. The tabulated data show that under small or moderate dosage (2 to 12 units daily) these very young hypophysectomized birds may begin crop-milk secretion within as little as 5 days, and they may also secrete at least as late as the 38th day. The effect of long-continued or of excessive stimulation of the crop-sacs of either normal or hypophysectomized pigeons is otherwise quite unknown.

Summary. In all of 20 pigeons completely hypophysectomized from 1 to 287 days earlier the crop-sacs were stimulated by prolactin to proliferation, and often to crop-milk formation within 4 days. These complete responses were obtained in both very immature and in adult individuals. Quantitative measurements made on the operated adults show, however, that their crop-sac response is only about one-eighth that of unoperated mature pigeons.

Complete adrenalectomy did not prevent the usual response of the crop-sacs to prolactin in a test started 4 days after operation. Thyroidectomy did not significantly affect the usual crop-sac response to prolactin in a pigeon operated 176 days earlier.

9250

Erythrocyte Count in Four Inbred Strains of Mice.

RALPH J. KAMENOFF. (Introduced by A. J. Goldforb.)

From the City College, New York City, and the Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, L. I.

Since wide variation in the erythrocyte count for adult mice *(Mus musculus)* appears in the literature, the problem of whether this variation is genetic arose. It also seemed desirable to establish a mean red cell count on a more adequate number of animals than usually reported.

The figures given by various authors for erythrocyte counts (in millions per cu. mm.) are as follows: Simmonds,[1] 6 to 8; Mixter and Hunt,[2] 9.7 for "non-flexed" based on 12 animals and 11.2 for "flexed" based on 16 animals; Kamenoff,[3] 9.9 for "non-flexed" based on 13 and 10.9 for "flexed" based on 5. The following figures are summarized from Scarborough[4] (in millions per cu. mm.):

1 Simmonds, J. P., *Anat. Record*, 1925, **30**, 99.

2 Mixter, R., and Hunt, H. R., *Genetics*, 1933, **18**, 367.

3 Kamenoff, R. J., *J. Morph.*, 1935, **58**, 117.

4 Scarborough, R. A., *Yale J. Biol. and Med.*, 1930, **3**, 272.

Author	Aver.	Maximum	Minimum	No. of animals
Goodall[5]	10.9	—	—	—
Klienenberger and Carl[6]	9.7	11.0	7.7	8
Klienenberger and Carl[6]	10.7	12.4	8.9	8
Lange[7]	9.3	—	—	—
Meyer[8]	8.3	—	—	—
Sundstroem[9]	10.1	—	—	10
Scarborough's average of these figures	9.7			

The work here reported was done in the laboratory of Dr. E. C. MacDowell of the Department of Genetics of the Carnegie Institution of Washington for which the author wishes to thank Dr. MacDowell, as well as for the use of his inbred mice strains.

The blood counts were made on samples of blood drawn from the tip of the tail and diluted 2, 3, or 4 to 1,000. Usually 2 counts were made from a single sample from each mouse. The mice were adult breeding stock in good condition and from 6 weeks to one year old. They were from 4 of the highly inbred strains maintained at the Carnegie Institution laboratory in Cold Spring Harbor. Each strain had been brother-sister inbred for over 30 generations, and were thus highly homozygous. The strains used were, first, an albino strain known as Bagg Albino (B. Alb.); second, a dilute brown strain (d. br.); third, a black strain known as C 58 (the strain used extensively by Dr. MacDowell in his leukemia work); and fourth, a pink eye dilute brown strain known as Storrs-Little (Sto-Li).

In Table I the mean, with its standard error, of each of the lines is given as well as the number of animals on which each figure is based. There is practically no difference between the means of the 4 strains. However, in all the strains the mean red count of the males is higher than that of the females, although possibly not significantly so in the C 58's. The mean for all males together, irrespective of strain, is 10,019,400 $\pm$ 68,900 and of the females 9,225,000 $\pm$ 46,700 with a difference of 794,431 $\pm$ 83,300, a significant difference. The mean for all 118 animals, irrespective of sex and strain, is 9,552,000 $\pm$ 58,700.

In Table II the red cell count for the extreme individual of each sex of each strain is given. Although the range in each strain is

[5] Goodall, A., *J. Path. and Bact.*, 1910, **14**, 195.

[6] Klienenberger, C., and Carl, W., *Die Blut-Morphologie der Laboratoriums-Tiere*, Leipzig, 1912.

[7] Lange, *Zool. Jahrb.*, 1919, **36**, 657.

[8] Meyer, S., *Folia hematol.*, 1924, **30**, 195.

[9] Sundstroem, E. S., *Am. J. Physiol.*, 1922, **60**, 443.

TABLE I.
Mean Erythrocyte Count of Several Strains (in corpuscles per cu. mm.)

Strain	No. of animals	Unweighted mean	Standard error	Weighted mean	No. of males	Mean of males	No. of females	Mean of females
B. Alb.	42	9,531,700	158,000	9,665,900	16	10,229,500	26	9,102,300
C 58	25	9,498,000	216,000	9,502,800	7	9,514,000	18	9,491,600
Sto-Li	26	9,515,400	233,000	9,563,800	11	9,881,000	15	9,246,600
d. br.	25	9,532,700	220,000	9,641,900	10	10,187,900	15	9,096,000
All	118	9,522,100	58,700	9,623,200	44	10,019,400	74	9,225,000

Unweighted mean is the mean based on all the animals of a given strain irrespective of sex.
Weighted mean is the mean obtained by giving equal weight to each sex irrespective of the number of individuals of each sex used.

TABLE II.
Extreme Erythrocyte Count in Several Strains (in corpuscles per cu.mm.).

Strain	High		Low	
	male	female	male	female
B. Alb.	11,893,000	10,660,000	8,580,000	7,455,000
C 58	10,110,000	11,657,000	9,013,000	6,693,000
Sto-Li	11,613,000	11,187,000	8,625,000	6,696,000
d. br.	11,507,000	10,487,000	8,608,000	7,600,000

extremely wide, the ranges of the different strains do not differ materially.

From these data, therefore, it must be concluded that these 4 strains do not have an inherited difference in the number of erythrocytes; and, since highly homozygous stock was used, the variations within each strain are not genetic but extrinsic in origin. Further, the average erythrocyte count of 9,700,000 per cu. mm. given in Scarborough, based on the work of 5 separate authors on an assumed total of 48 animals, does not differ widely from the count of 9,550,000 per cu. mm. based on 118 animals in this study.

9251

Experimental Fever Therapy in Myxomatosis and Fibroma of Rabbits.*

EARL B. MCKINLEY AND ELLEN G. ACREE.

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We have recently attempted to test the effect of induced fever on the development of myxomatosis and fibroma in rabbits. There are a large number of fever machines[1] in use in clinics and in offices of practicing physicians, and the subject of fever therapy in various pathological conditions is growing rapidly in importance. In some of these, notably in the acute pelvic inflammation due to *Neisseria gonorrhœæ,* the treatment has proved its efficiency and worth. In other conditions fever therapy is employed experimentally, or empirically, as in the case of arthritis. Clinical results are good, bad, and indifferent, depending upon the individual patient and his dis-

* Appreciation is expressed to the General Electric X-Ray Corporation for its courtesy in supplying us its Inductotherm for experimental use.

[1] Krusen, Frank H., *J. Am. Med. Assn.,* 1936, **107**, 1215.

ease. While this type of therapy has been directed for the most part toward bacterial inflammatory conditions, little has been done with the virus diseases. The authors, therefore, have attempted to set up crucial experiments with the viruses of myxomatosis and fibroma in order to determine if induced fever would affect these virus processes in any way.

In the first place, we selected the Inductotherm apparatus manufactured by the General Electric X-Ray Corporation[2, 3] as one suitable for working with small animals such as rabbits, and for convenience in the laboratory. The principle of this apparatus is that of electromagnetic induction, with vacuum tube oscillator which generates an alternating current of 12,000,000 cycles per second. Such a device produces heat in electrolytes in direct proportion to the electrical conductivity of such electrolytes and should produce heat in living tissue in proportion to the vascularity of the tissue.

In testing this heating apparatus on normal rabbits, we found that the animals withstood rather prolonged heating if gradually accustomed to it, but rapid heating for even 10 to 20 minutes frequently killed the animals. Temperatures as high as 107°F. to 110°F. could be reached in from 10 to 40 minutes, but the animals did not survive. On the other hand, temperatures could be raised gradually to 106°F. or 107°F. within 5 to 10 minutes, and fever would persist in the animal for a couple of hours or more without any ill effects. When the temperature fell, it could be gradually raised again and the animal survived.

After learning the limitations of the apparatus and something of the resistance of normal rabbits, we tested the effect of rather prolonged and repeated fever on animals infected with the viruses of myxomatosis and rabbit fibroma. At first these virus tumors were allowed to develop to clinical proportions before fever therapy was begun. Under these conditions, the fever therapy had no effect at all on the progress of the disease process. Gradually the time was shortened until we began fever therapy immediately following injection of the animals with the viruses. The experiment was divided into 2 groups. The first group was given a single 10-minute heating period daily, and the second group 2, 3, or, when the animal's condition permitted, 4 heating periods daily. Occasionally it was necessary to lessen the heating time in order to save the animal since the temperature continued to rise even after the current was turned off. In some instances the maximum temperature was not

2 Council on Physical Therapy, *J. Am. Med. Assn.*, 1935, **104**, 1706.

3 Council on Physical Therapy, *J. Am. Med. Assn.*, 1936, **106**, 1091.

reached until 15 to 18 minutes later. Between heatings the temperature was allowed to drop gradually to a point sufficiently low for further treatment. The return to normal temperature required from an hour to an hour and a half. In myxomatous animals, the diseased condition was so advanced after 6 days that treatment was discontinued and death occurred from 2 to 3 days later. In animals infected with fibroma, daily treatments over a period of 2 weeks did not retard the progress of the disease. Again fever therapy had no effect upon the ultimate development of these 2 virus diseases in the animals.

While our report is, therefore, of a negative nature, it is important perhaps to point out that, under the conditions of our experiments, and with the 2 viruses mentioned, fever therapy is apparently without value. In the cases of these 2 viruses, therefore, we have contrast with certain bacterial infections (such as *Neisseria gonorrheæ*) where fever therapy has been found efficacious. It would seem that at least some viruses are more resistant to induced high temperatures than are some bacterial forms.

9252 P

Effect of Estrogenic Substances in *Lebistes reticulatus* (Guppy).

PHILIP BERKOWITZ. (Introduced by Harry A. Charipper.)

From the Department of Biology, Washington Square College, New York University.

The adult male *Lebistes reticulatus* possesses, as its secondary sex characters, a gonopod (intromittent organ which is the anal fin in the female, and in young fish of both sexes) and various color patterns. The adult female possesses no such gonopod or colorations, but is much larger than the male. The average length of the male (from tip of snout to base of caudal fin) is approximately 18 mm.; that for the adult female is about 30 mm. (Goodrich, *et al.*,[1] confirmed by present observations). These differentiating secondary sex characters are not present at birth, at which time both male and female are identical in appearance. At about 35 days after birth there is a divergence in size of the 2 sexes, from which time the females normally become larger than the males. The gonopod of the male makes

[1] Goodrich, H. B., Dee, J. E., Flynn, C. M., and Mercer, R. N., *Biol. Bull.*, 1934, **67**, 83.

its first appearance at approximately 40 days, and the colorations from 50-60 days after birth.

In view of the scarcity of information concerning hormonic influences on these and related processes in the fish, an attempt has been begun, under the direction of Dr. Robert Gaunt,* to analyze such influences in the guppy. The first phase of this study, concerned with the effect of estrogenic substances, is here reported.

Sixty fish have been treated with Progynon tablets.† These tablets contained 45 R. U. of estrogenic substance, active by mouth in mammals, the exact chemical nature of which was not known. When crumbled into the aquaria (one tablet per 2-gallon tank, 3 times a week) the fish ate the tablets freely. The fish were fed on this hormone from birth for periods of one to 5 months. No male secondary sex characters appeared during the period of treatment although the fish had reached the size and age at which sexual differentiation should have been completed. The size and shape of these fish approached that of the female sex; the coloring and condition of the anal fin were entirely those of the female.

Fourteen fish were treated for 2 months and then placed in tanks with fresh water. Eight assumed male secondary sex characters 40 days after discontinuance of the hormone, *i. e.,* a period closely corresponding to the time necessary for normal males to attain sexual differentiation from birth. The absence of male secondary sex characters during treatment is not due to slow growth resulting from unfavorable conditions of treatment, for all treated males outgrew their controls and went well above the normal size for males. The average length of treated males was 26 mm. as compared to 18 mm. for controls. When hormone was discontinued treated males reverted to normal as regards the gonopod and the color conditions, but they did not revert to normal male size.

Of 5 fish treated for 4-5 months, all showed external sex characters of the female type. Four were killed; 3 possessed ovaries, and 2 also had young. The fourth had a gonad definitely not normal for either sex.

Examination of this gonad and the testes of other males treated for shorter periods showed a distinct effect of hormone treatment. There was a considerable suppression of spermatogenesis, and a relative increase in the number of young germ cells. In the latter

* The author is grateful for the advice and suggestions of Drs. H. A. Charipper and M. H. Harnly.

† The author is indebted to Dr. Gregory Stragnell of the Schering Corp., for supplying the Progynon tablets; to Mr. C. M. Breder of the New York Aquarium, and to Mr. H. E. Potts for animals and advice as to their proper care.

regard, an immature condition was suggested. Sperm ducts were apparently stimulated to increased growth by the hormone. The spermatogenic suppression might have been due either to a direct effect of estrogens on the male gonad or to an inhibition of hypophyseal activity. The factors involved are being studied further.

The secondary sex characters of adult males could not be modified by estrogenic hormone treatment beginning at maturity and continued for periods up to 4 months.

Conclusions. Estrogenic hormone feeding, started at birth, caused the suppression of male secondary sex characters and a marked suppression of spermatogenesis in the male guppy.

9253 P

Release of Spermatozoa by Anterior Pituitary Treatment of the Male Frog, *Rana pipiens.*

ROBERTS RUGH.

From the Zoology Department, Columbia University.

Houssay and Lascano-Gonzalez,[1] using *Bufo marinus* males, demonstrated that hypophyseal removal causes degeneration and hypophyseal implantation causes hypertrophy of the testes. O. M. Wolfe[2] implanted pituitary glands subcutaneously to induce amplexus in *Rana pipiens* and Rugh[3] showed that extracts of mammalian anterior pituitary (antuitrin-S or whole sheep gland) would induce amplexus in toads but not in frogs.

The standard technique for securing developing frog's eggs[4] does not require pituitary treatment of males. Functional sperm are available at all times during hibernation simply by cutting up the testes of mature, hibernating frogs in spring water. However, anterior pituitary injection of hibernating male frogs not only induces amplexus (with ovulating females of the same species, only) but releases from the Sertoli cells all mature spermatozoa. This can be demonstrated by subjecting hibernating males from which single testes have been removed to anterior pituitary treatment. Amplexus

1 Houssay, B. A., and Lascano-Gonzalez, J. M., *Compt. rend. Soc. biol.*, 1929, **101**, 938.

2 Wolfe, O. M., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 692.

3 Rugh, R., *Biol. Bull.*, 1935, **68**, 74.

4 Rugh, R., *Biol. Bull.*, 1934, **66**, 22.

will be achieved in about 16 hours and the remaining testis may then be removed and studied. In the control testis (Fig. 1) the mature sperm may be seen clustered in groups around their Sertoli cells while the testis from the same frog after sexual stimulation by

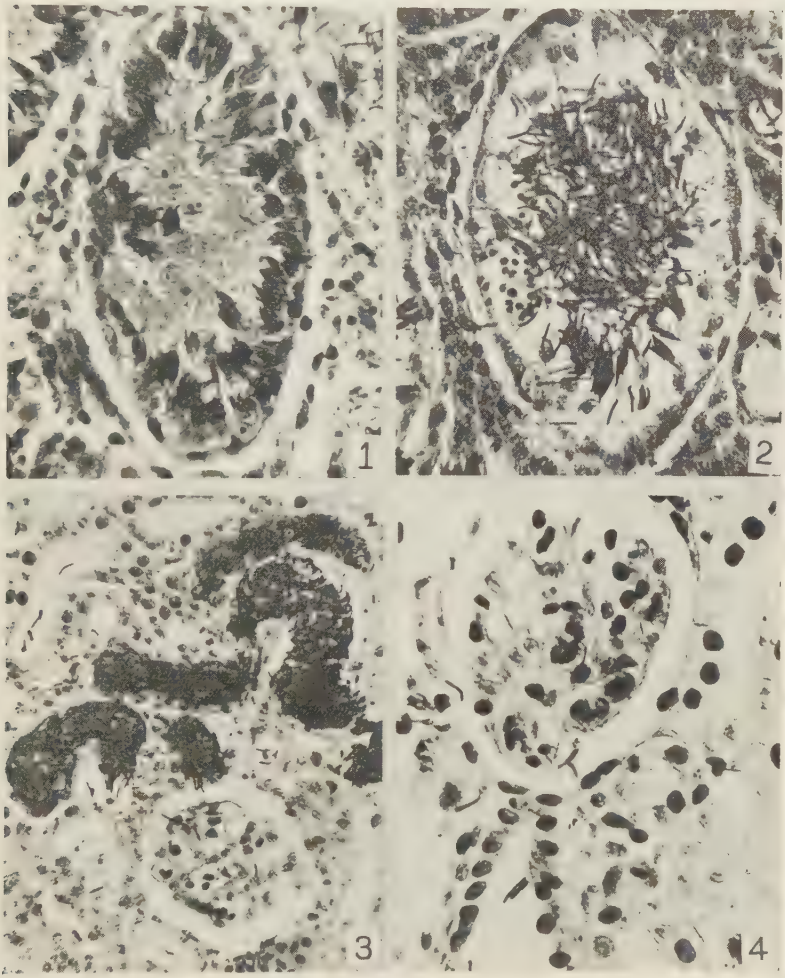

FIG. 1.
Seminiferous tubule from the right testis of an hibernating frog.

FIG. 2.
Seminiferous tubule from the left testis of the same frog (Fig. 1) 16 hours after the frog was injected with anterior pituitary hormone.

FIG. 3.
Section of kidney of frog 16 hours after anterior pituitary injection showing tubules filled with spermatozoa.

FIG. 4.
Highly magnified section of kidney showing Bowman's capsule and connecting uriniferous tubule, both containing spermatozoa.

anterior pituitary injection shows all of the mature sperm liberated into the lumen of the seminiferous tubule (Fig. 2). This effect can be achieved only by anterior pituitary treatment, the controls having received other frog organs and having been subjected to temperature changes from 4°C. to 28°C. The reaction is comparable to the follicle changes induced in females by anterior pituitary treatment[5] except that in the female the egg is released into the body cavity.

In sections of the kidney adjacent to the stimulated testis, spermatozoa may be seen in the uriniferous tubules, Bowman's capsule, and ureter (Figs. 3 and 4). The exact path of these spermatozoa, through the kidney, is being worked out for *Rana pipiens, Rana catesbiana, Bufo fowleri,* and *Hyla crucifer.*

Studies are at present being made to determine the source of the male gonad hormone, and the seasonal differences in maturation exhibited by *Rana pipiens* and *Rana catesbiana.* In the one there is a single breeding period, with a single expulsion of spermatozoa. In the other there seems to be an extended breeding period, at a different time of the year, with staggered maturation of spermatozoa.

9254

Water Intake and the Blood Sugar Level.

M. CAROLINE HRUBETZ. (Introduced by H. B. Williams.)

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In our studies which have involved blood sugar determinations on rats, considerable effort has been expended to secure uniformity both in the animal material and in the routine care of the colony. But in spite of all this care to secure uniformity we have found at times that the normal blood sugar level showed considerable variation.[1] Recently, upon our return from the summer vacation, the routine normal control series of sugar determinations was made. At the same time, determinations were made upon a group of rats used in the study of prolonged high carbohydrate feeding.[2] These animals represented the 4th, 5th and 6th generations which had been kept continuously on the high carbohydrate diet. It was noticed that the sugar levels in both groups were not only high but far above

5 Rugh, R., *J. Exp. Zool.*, 1935, **71**, 163.

1 Hrubetz, M. C., *J. Biol. Chem.*, 1934, **107**, 731.

2 Hrubetz, M. C., *J. Lab. Clin. Med.*, 1936, **21**, 1142.

any mean value thus far obtained. Two and a half months previously the water supply had been changed from the open jar to the tubulated-bottle system. It had been observed that some of the water bottles did not deliver properly. The question arose, therefore, as to whether a possible water deficiency due to difficult access to the water by the tubulated-bottle method was associated with the high sugar level. The purpose of the observations to be reported was to determine the influence, if any, of the water consumption by the open jar and the tubulated bottle upon the blood sugar level.

After the initial observations were made, the entire colony was supplied with open water jars and the blood sugar levels were determined one week later and at subsequent intervals for 8 weeks. Approximately 50 observations were made on each point on the curve for the normal controls. There were 30 animals in the high carbohydrate group. The determinations were made by the Somogyi Micro Method[3] with the use of reagent No. 1. The chart shows a prompt drop in the normal animals to a level very near the mean for the colony. The individual values fluctuated rather widely the second week and then remained constant throughout the remainder of the 8 weeks period. The high carbohydrate rats maintained a high blood sugar for 3 weeks; not until the 4th week did they return to a normal level. After the return, however, the level remained constant for the remainder of the period.

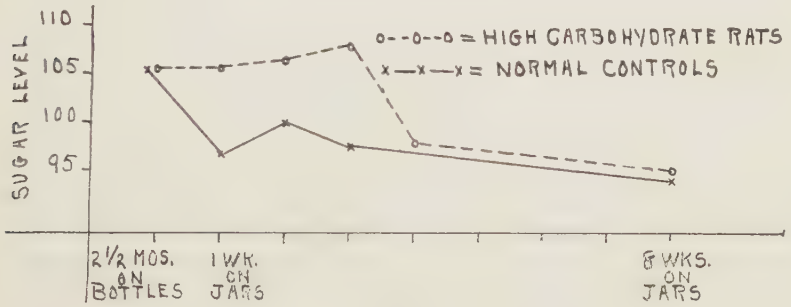

FIG. 1.

Naturally, we were somewhat surprised to find this increase associated with water intake and at present are quite unable to suggest its mechanism. It may possibly be associated with the general concentration of the blood though no measurements were made to determine this. Another point worthy of note and for which we have at present no information was the delayed return to the normal by

3 Peters and Van Slyke, *Quantitative Clinical Chemistry*, Williams and Wilkins Co., Baltimore, 1935, Vol. II, p. 466.

the animals on the high carbohydrate diet. Apparently, diet is a factor influencing the ability of the organism in maintaining a more or less constant concentration of the blood as well as of some of its specific constituents. In spite of our inability at present to suggest the mechanism involved in the reactions reported, the practical bearing on standardization of experimental material is of sufficient importance to justify prompt publication.

9255 P

Determination of Sulfanilamide in Blood and Urine.

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From the Department of Pharmacology and Experimental Therapeutics, The Johns Hopkins University.

Recently a method was described for the estimation of sulfanilamide (para-aminobenzenesulfonamide) in blood and urine.[1] In the rabbit and human subject this drug is partly excreted in the form of an acetylated derivative.[2] In developing a method for determining this conjugated derivative in blood, we have been able to improve the original procedure.

The present modification possesses the advantages of being more sensitive, of giving a more stable color, and of requiring fewer manipulations. Sulfanilamide can be determined very accurately by this method, and in addition one can obtain a fairly good estimate of the conjugated form present in blood. The present method consists of preparing a blood filtrate with toluenesulfonic acid, utilizing the acidity of the precipitant to perform diazotization and coupling with the amine. In determining the conjugated sulfanilamide in blood, the acidity of the blood filtrate is sufficient for hydrolysis on heating.

One volume of oxalated blood is measured into a flask, diluted and laked with 7 volumes of 0.05% saponin solution.* After laking is complete (1 or 2 minutes) 2 volumes of para-toluenesulfonic acid

[1] Marshall, E. K., Jr., Emerson, Kendall, Jr., and Cutting, W. C., *J. A. M. A.*, 1937, **108**, 953.

[2] Marshall, E. K., Jr., Cutting, W. C., and Emerson, Kendall, Jr., *Science*, 1937, **85**, 202.

* Laking can be accomplished by diluting with water instead of saponin solution at least 15 minutes before adding the protein precipitant.

solution (20 gm. dissolved in water and diluted to 100 cc.) are added with shaking. After 5 minutes' standing, the mixture is filtered, and 10 cc. of the clear filtrate (a smaller amount can be used with proportionate reduction of the nitrite and dimethyl-α-naphthylamine reagents) is measured into a small flask and 1 cc. of 0.1% freshly prepared† sodium nitrite solution is added. After 3 minutes' standing, 5 cc. of a solution of dimethyl-α-naphthylamine (1 cc. in 250 cc. of 95% ethyl alcohol) is added from a burette. Ten cc. of a standard solution of sulfanilamide containing toluenesulfonic acid (18 cc. of the 20% solution per 100 cc.) is measured into a flask and treated like the blood filtrate. A 1 mg. % standard is satisfactory for bloods containing from 5 to 20 mg. % of sulfanilamide. The solutions can be compared in a colorimeter at any time from 10 to 60 minutes after the naphthylamine has been added, so that several blood filtrates can be read against one standard.

To determine the conjugated sulfanilamide (probably paraacetylbenzenesulfonamide) in blood, 10 cc. of the toluenesulfonic acid blood filtrate is placed in a tube graduated at 10 cc. and heated in a boiling water bath for 90 minutes. The solution is then cooled and diluted to 10 cc. to replace water loss by evaporation. The procedure is now exactly the same as that used in determining free sulfanilamide. The difference between the values obtained after and before hydrolysis gives the amount of conjugated compound (calculated as sulfanilamide).

If a determination of the sulfanilamide only is desired and toluenesulfonic acid is not available one can use trichloracetic acid to precipitate the blood proteins. One volume of blood is measured into a flask, diluted and laked with 8 volumes of 0.05% saponin solution, and precipitated by adding one volume of 20% trichloracetic acid. The filtrate is treated exactly like that obtained with toluenesulfonic acid, and the standard contains 8 cc. of 20% trichloracetic acid per 100 cc.

For the estimation of the sulfanilamide content of the blood of patients receiving the drug, plasma or serum can be used instead of whole blood as there is no essential difference in the values obtained.

† We prepare the nitrite solution fresh each day. It is advisable to use a very pure sodium nitrite. We have found that Kahlbaum's sodium nitrite obtained from Akatos in New York is satisfactory. We have purchased the dimethyl-α-naphthylamine (No. 1060) and the para-toluenesulfonic acid (No. 984) from the Eastman Kodak Company, Rochester, New York. As stated before it is essential to use all glass cups in the colorimetric comparison as the ordinary cup with a detachable glass bottom in a metal frame may give inconsistent and erroneous results.

Urine is diluted so that it contains about one mg. % of sulfanilamide. Ten cc. of this diluted urine is measured into a flask, one cc. of 20% toluenesulfonic acid added, and then one cc. of 0.1% sodium nitrite. After 3 minutes, 5 cc. of dimethyl-α-naphthylamine solution (1 cc. in 250 cc. of 95% ethyl alcohol) are added. A standard solution of sulfanilamide is treated in the same way. After 5 to 10 minutes, the solutions can be compared in the colorimeter. To determine the acetyl-derivative in urine, one cc. of urine is mixed with 2 cc. of N hydrochloric acid, heated in a boiling water bath for 30 minutes, neutralized with 2 N sodium hydroxide, and diluted to appropriate volume. The determination of total sulfanilamide is then made as described above.

9256 P

Neoplasms in Albino Rats Resulting from the Feeding of Crude Wheat Germ Oil Made by Ether-Extraction.

L. G. ROWNTREE, JOHN LANSBURY AND ARTHUR STEINBERG.

From the Philadelphia Institute for Medical Research in the Philadelphia General Hospital.*

The effectiveness of thymus and pineal extract in controlling the rate of growth and development in the young when administered through successive generations of rats led us to attempt the same procedure with the various vitamins. In administering vitamin E we have employed an ether-extracted crude wheat germ oil† prepared by the extraction of fresh wheat germ with ether. All animals fed this preparation of wheat germ oil eventually developed tumors.

The rats used were placed on the stock diet of the Institute, containing the following ingredients: rolled oats 15 parts, cracked corn 60, dried meat scraps 14, powdered milk 10, sodium chloride 1. To each 100 gm. was added 1.25 cc. of cod liver oil. Additional nutriment consisted of green vegetables once a week.

From July 3, 1934, four animals were fed one cc. daily of wheat germ oil, the oil being mixed with the food. On October 29, 1934,

* From the Samuel Bell, Jr., Laboratory.

† This oil was especially prepared for us and kindly donated by E. R. Squibb & Sons. It is not their refined wheat germ oil (Zygon) designed for human use. We wish to express our appreciation to Dr. John F. Anderson for his cooperation in preparing this special oil, and in other ways.

116 days having elapsed, nodules were palpable in the abdomen of 2 of these rats. The rats were killed on November 12, 1934, and pieces of the resultant solid tumors present in the abdomen were transplanted to 2 other rats of the same strain. The grafts grew in both animals. The other 2 animals which were fed with the oil developed tumors 2 months later. These growths have been transplanted through 6 generations.

To check this result 10 albino rats (Wistar strain) were fed about one cc. per rat per day of crude wheat germ oil, beginning November 29, 1934. The only change was the addition to the food of wheat germ oil. Within the next 200 days tumors were palpable in the abdominal cavity of the 10 rats. In one case the tumor weighed 44 gm. In another animal 3 tumors were present, 2 arising apparently from the distal ends of the uterine horns and one from the mesentery. A certain amount of necrosis was not infrequent. These tumors were readily transplantable in the Wistar strain stock animals.

The malignant nature of these tumors is demonstrated by the fatal outcome, the morphology of the tumor cells and the inoculability through 6 generations. The implants grow with great rapidity, attaining a large size and result in the death of the animals. In many instances after the tumor had attained the size of 3x3 cm., it underwent necrosis and ulceration.

Microscopically both the primary and transplanted tumors are composed chiefly of spindle cells. In some areas the cells are small, compact and closely packed, with narrow elongated nuclei; in others the individual cells are larger and separated by fibrous tissue with large hyperchromatic nuclei, frequently showing mitoses. The cellular areas of the tumors and the evidence of rapid proliferation suggest a sarcomatous nature.

Negative results have been obtained to date from the feeding of refined ether-extracted wheat germ oil (Squibb), gasoline-extracted wheat germ oil (Squibb), benzine-extracted wheat germ oil (Squibb), Triticol (an expressed oil, kindly supplied by the Pharmaceutical Specialties Company), certified compressed wheat germ oil and vitamin E concentrate (furnished by General Mills and administered during the last 5 to 7 weeks). Linseed oil, sesame oil, cod liver oil, oleic acid and caritol have also yielded negative results.

Conclusions. Fourteen rats fed with unrefined wheat germ oil which contained the sediment deposited therefrom developed abdominal tumors. The neoplasm following the ingestion of oil is malignant in nature and microscopically a sarcoma. It is readily trans-

plantable and retains its malignancy through 6 successive implantations.‡

9257

Production of Sarcoma in Albino Rats as a Result of Feeding Crude Wheat Germ Oil.

G. M. DORRANCE* AND E. F. CICCONE.* (Introduced by L. G. Rowntree.)

From the Philadelphia Institute for Medical Research.

Subsequent to the work of Rowntree, Lansbury, Steinberg and Anderson,[1] † in which 14 tumors had been observed in 14 rats fed with a crude preparation of ether-extracted wheat germ oil, the Oncologic Hospital group was asked to elaborate this work and to determine and establish the nature of the tumor.

It was found that by increasing the amount of oil fed, the tumor could be produced as early as 60 days. Thus in 34 albino rats of the Wistar and Buffalo strains fed crude wheat germ oil, 100% developed intraäbdominal tumors. These tumors have been found in the omentum, in the mesentery, in both small and large intestines, in the area of the uterus and subperitoneally. Many animals have developed multiple tumors in the abdominal cavity. The point of origin thus far has been consistent only in its having been found in the peritoneal cavity. The size of the tumors depends, to some extent, on the duration of the time before the rat is sacrificed. The tumors have been as large as 3x3 cm. There has been no lymphatic extension or metastasis to nodes. The only instance in which another organ has been involved was in one case with direct extension to the liver. If not sacrificed, the tumor becomes large enough to cause death often by intestinal obstruction.

In the Wistar strain, 22 tumors have been successfully transplanted to date. No attempt has been made to transplant the others of this group of tumors, as the animals were found dead in the

‡ We wish to express our appreciation to Drs. J. H. Clark, Joseph McFarland, and Francis Carter Wood, all of whom agree that this tumor is sarcomatous in nature.

* The American Oncologic Hospital.

1 Rowntree, Lansbury, Steinberg and Anderson, PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 424.

† Neoplasms resulting from the feeding of ether-extracted, crude wheat germ oil.

cage. Of the 260 rats receiving implants in this group, 100% resulted in successful takes and many of these tumors have been retransplanted through 15 successive recipients. If the implants were made intraperitoneally instead of subcutaneously, they grew more readily and did not ulcerate.

Histologically and clinically the tumors have all the characteristics of spindle cell sarcoma.

Summary. Sarcomas were produced in all 34 rats by feeding crude wheat germ oil. Tumors were produced in both the Philadelphia Institute for Medical Research and in the American Oncologic Hospital. With increasing amounts of oil the time required for the development of tumors was decreased. These tumors have been successfully transplanted both subcutaneously and intraperitoneally in 260 rats through 15 successive generations. These tumors are all spindle cell sarcomas.

ERRATA

Article in the March, 1937, number, by C. R. Schmidt and A. C. Ivy, page 91, table 2, should read

$$\frac{\text{Common Duct Diameter}}{\text{Body Weight}}$$

Effect of Freezing on Transmitting Agent of Neoplasms, by C. Breedis, W. A. Barnes and J. Furth, the abscissa, page 223, should read seconds, not minutes.

Article 9192, page 259, should read, "Studies in the Physiology of Blood," etc.